Chittaranjan Kole *Editor*

Genomic Designing of Climate-Smart Vegetable Crops

Springer

Genomic Designing of Climate-Smart Vegetable Crops

Chittaranjan Kole
Editor

Genomic Designing of Climate-Smart Vegetable Crops

Springer

Editor
Chittaranjan Kole
Department of Atomic Energy
Government of India, ICAR-National
Institute for Plant Biotechnology
New Delhi, India

ISBN 978-3-319-97414-9 ISBN 978-3-319-97415-6 (eBook)
https://doi.org/10.1007/978-3-319-97415-6

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Dedicated to

Late Prof. Subir Sen, D.Sc.

Head, Department of Genetics & Plant Breeding, Faculty of Agriculture, and Dean, Post-Graduate Studies, Bidhan Chandra Krishi Viswavidyalaya (Agricultural University), West Bengal, India

An exceptional scientist, outstanding academician and a visionary

who lived many decades ahead of his time

Preface

The last one hundred and twenty years have witnessed a remarkable evolution in the science and art of plant breeding culminating in quite a revolution in the second decade of the twenty-first century! A number of novel concepts, strategies, techniques and tools have emerged from time to time over this period and some of them deserve to be termed as milestones. Traditional plant breeding, immediately following the rediscovery of laws of inheritance, has been playing a spectacular role in the development of innumerable varieties in almost all crops during this entire period. Mention must be made on the corn hybrids, rust-resistant wheat, and obviously the high-yielding varieties in wheat and rice that ushered the so-called green revolution. However, the methods of selection, hybridization, mutation and polyploidy employed in traditional breeding during this period relied solely on the perceivable phenotypic characters. But most, if not all, of the economic characters in crops are governed by polygenes which are highly influenced by environment fluctuations, and hence phenotype-based breeding for these traits has hardly been effective.

Historical discovery of DNA structure and replication in 1953 was followed by a series of discoveries in the 1960s and 1970s that paved the way for recombinant DNA technology in 1973 facilitating the detection of a number of DNA markers in 1980 onwards and their utilization in construction of genetic linkage maps and mapping of genes governing the simply inherited traits and quantitative trait loci controlling the polygenic characters in a series of crop plants starting with tomato, maize and rice. Thus new crop improvement technique called as molecular breeding started in later part of the twentieth century. On the other hand, genetic engineering made modification of crops for target traits by transferring alien genes, for example, the *Bt* gene from the bacteria *Bacillus thuringiensis*. A large number of genetically modified crop varieties have thus been developed starting with the commercialization of 'flavr Savr' tomato in 1994.

Meantime, the manual DNA sequencing methodology of 1977 was being improved with regard to speed, cost-effectiveness and automation. The first-generation sequencing technology led to the whole genome sequencing of Arabidopsis in 2000 and followed by rice in 2002. The next-generation sequencing technologies were available over time and used for sequencing of genomes of many

other models and crop plants. Genomes, both nuclear and organellar, of more than 100 plants have already been sequenced by now and the information thus generated are available in public database for most of them. It must be mentioned here that bioinformatics played a remarkable role in handling the enormous data being produced in each and every minute. It can be safely told that the 'genomics' era started in the beginning of the twenty-first century itself accompanying also proteomics, metabolomics, transcriptomics and several other 'omics' technologies.

Structural genomics have thus facilitated annotation of genes, enumeration of gene families and repetitive elements, and comparative genomics studies across taxa. On the other hand, functional genomics paved the way for deciphering the precise biochemistry of gene function through transcription and translation pathways. Today, genotyping-by-sequencing of primary, secondary and even tertiary gene pools; genomewide association studies; and genomics-aided breeding are almost routine techniques for crop improvement. Genomic selection in crops is another reality today. Elucidation of the chemical nature of crop chromosomes has now opened up a new frontier for genome editing that is expected to lead the crop improvement approaches in near future.

At the same time, we will look forward to the replacement of genetically modified crops by cisgenic crops through transfer of useful plant genes and atomically modified crops by employing nanotechnology that will hopefully be universally accepted for commercialization owing to their human-friendly and environment-friendly nature.

I wish to emphatically mention here that none of the technologies and tools of plant breeding is too obsolete or too independent. They will always remain pertinent individually or as complimentary to each other, and will be employed depending on the evolutionary status of the crop genomes, the genetic resources and genomics resources available, and above all the cost-benefit ratios for adopting one or more technologies or tools. In brief, utilization of these crop improvement techniques would vary over time, space and economy scales! However, as we stand today, we have all the concepts, strategies, techniques and tools in our arsenal to practice genome designing, as I would prefer to term it, of crop plants not just genetic improvement to address simultaneously food, nutrition, energy and environment security, briefly the FNEE security, I have been talking about for the last 5 years at different platforms.

Addressing FNEE security has become more relevant today in the changing scenario of climate change and global warming. Climate change will lead to greenhouse gas emissions and extreme temperatures leading to different abiotic stresses including drought or waterlogging on one hand and severe winter and freezing on the other. It will also severely affect uptake and bioavailability of water and plant nutrients and will adversely cause damage to physical, chemical and biological properties of soil and water in cropping fields and around. It is also highly likely that there will be emergence of new insects and their biotypes and of new plant pathogens and their pathotypes. The most serious concerns are, however, the unpredictable crop growth conditions and the unexpected complex interactions among all the above stress factors leading to drastic reduction in crop yield and

quality in an adverse ecosystem and environment. Climate change is predicted to significantly reduce productivity in almost all crops. For example, in cereal crops the decline of yield is projected at 12–15%. On the other hand, crop production has to be increased at least by 70% to feed the alarmingly growing world population, projected at about 9.0 billion by 2050 by even a moderate estimate.

Hence, the unpredictability of crop growing conditions and thereby the complexity of biotic and abiotic stresses warrant completely different strategies of crop production from those practiced over a century aiming mostly at one or the few breeding objectives at a time such as yield, quality, resistance to biotic stresses due to disease-pests, tolerance to abiotic stresses due to drought, heat, cold, flood, salinity, acidity or improved water and nutrient use efficiency. In the changing scenario of climate change, for sustainable crop production, precise prediction of the above limiting factors by long-term survey and timely sensing through biotic agents and engineering devices and regular soil and water remediation will play a big role in agriculture. We have been discussing on 'mitigation' and 'adaptation' strategies for the last few years to reduce the chances of reduction of crop productivity and improve the genome plasticity of crop plants that could thrive and perform considerably well in a wide range of growing conditions over time and space. This is the precise reason of adopting genomic designing of crop plants to improve their adaptability by developing climate-smart or climate-resilient genotypes.

Keeping all these in mind, I planned to present deliberations on the problems, priorities, potentials and prospects of genome designing for development of climate-smart crops in about 50 chapters, each devoted to a major crop or a crop group, allocated under five volumes on cereal, oilseed, pulse, fruit and vegetable crops. These chapters have been authored by more than 250 of eminent scientists from over 30 countries including Argentina, Australia, Bangladesh, Belgium, Brazil, Canada, China, Egypt, Ethiopia, France, Germany, Greece, India, Ireland, Japan, Malaysia, Mexico, New Zealand, Kenya, Pakistan, Philippines, Portugal, Puerto Rico, Serbia, Spain, Sri Lanka, Sweden, Taiwan, Tanzania, Tunisia, Uganda, UK, USA and Zimbabwe.

There are a huge number of books and reviews on traditional breeding, molecular breeding, genetic engineering, nanotechnology, genomics-aided breeding and gene editing with crop-wise and trait-wise deliberations on crop genetic improvement including over 100 books edited by me since 2006. However, I believe the present five book volumes will hopefully provide a comprehensive enumeration on the requirement, achievements and future prospects of genome designing for climate-smart crops and will be useful to students, teaching faculties and scientists in the academia and also to the related industries. Besides, public and private funding agencies, policy making bodies and the social activists will also get a clear idea on the road travelled so far and the future roadmap of crop improvement.

I must confess that it has been quite a difficult task for me to study critically the different concepts, strategies, techniques and tools of plant breeding practiced over the last 12 decades that also on a diverse crop plants to gain confidence to edit the chapters authored by the scientists with expertise on the particular crops or crop groups and present them in a lucid manner with more or less uniform outline of

contents and formats. However, my experience gained over the last 7 years in the capacity of the Founding Principal Coordinator of the International Climate-Resilient Crop Genomics Consortium (ICRCGC) was highly useful while editing these books. I have the opportunity to interact with a number of leading scientists from all over the world almost on a regular basis. Organizing and chairing the annual workshops of ICRCGC since 2012 and representing ICRCGC in many other scientific meetings on climate change agriculture offered me a scope to learn from a large number of people from different backgrounds including academia, industries, policymaking and funding agencies and social workers. I must acknowledge here the assistance I received from all of them to keep me as a sincere student of agriculture specifically plant breeding.

This volume entitled Genomic Designing of Climate-Smart Vegetable Crops includes eight major crops including Potato, Tomato, Brassica Vegetables, Eggplant, Capsicum, Carrot, Alliums and Garlic. These chapters have been authored by 32 scientists from 9 countries including Argentina, Bangladesh, China, France, India, Japan, Poland, UK and USA. I place on record my thanks for these scientists for their contributions and cooperation.

I have always enjoyed working on horticultural crops during my entire academic career spanning over 40 years. I worked on molecular genetics and breeding in tomato while at the Pennsylvania State University, USA; molecular genetics, breeding and genomics in peach, apricot and bitter melon while at the Clemson University, USA; molecular genetics in country bean while at the Odisha University of Agriculture & Technology, India; molecular genetics in guava while at the Sam Higginbottom University of Agriculture, technology & Sciences, India; and molecular genetics and breeding in bitter melon while at the Bidhan Chandra Krishiviswavidyalaya (Agricultural University), and ICAR-National Institute for Plant Biotechnology, both in India.

However, I started working on horticultural crops in late seventies in the laboratory of (Late) Prof. Subir Sen Head of the Department of Genetics and Plant Breeding and later on Dean of Post-Graduate Studies in the Bidhan Chandra Krishiviswavidyalaya (Agricultural University), West Bengal, India as a Ph.D. student on genetics and breeding of a medicinal and aromatic plant, citronella. It is that time, we realized the potential of medicinal and aromatic plants as 'crops' in future and importance of exploration, collection, conservation, characterization and utilization of such crops the concepts that have become important in today's world. We are coming often across the terms 'biodiversity', 'health security' and 'crops of the future' only now! Prof. Sen was not only an outstanding scientist and an excellent teacher himself but also a visionary endowed with vast knowledge on arts, music and literature who lived many decades ahead of his time. Hence, I have dedicated this book to (Late) Prof. Sen as a token of my respect, appreciation and gratitude.

New Delhi, India Chittaranjan Kole

Contents

Abbreviations

$\bullet O_2^-$	Superoxide radical
•OH	Hydroxyl radical
5-azaC	5-Azacitidine
6-PF-2-K/Fru2,6-P_2ase	6-Phosphofructo-2-kinase/fructose2,6-bisphosphatase
ABA	Abscisic acid
ABF	ABA binding factor
ABRE	ABA-responsive element
ACC	1-Aminocyclopropane-1-carboxylic acid
ACS	*A. chinese* saponins
AFLP	Amplified fragment length polymorphism
AGO	Argonaute
AIR	Anthocyanin-impaired-response
AOX	Alternative oxidase
AP2	Apetala 2
AREB	ABA-responsive element binding protein
ASE	Allele-specific expression
ASH1	Absent, small or homeotic disks 1
ASHH2	ASH1 homolog 2
ATX	ARABIDOPSIS TRITHORAX
ATXR	ARABIDOPSIS TRITHORAX-RELATED
AVRDC	World Vegetable and Development Center (Tainan, Taiwan)
BAC	Bacterial artificial chromosome
BADH	Betaine aldehyde dehydrogenase
BC	Backcross
bHLH	Basic helix-loop-helix
BiFC	Bimolecular fluorescence complement
BPH	Best-parent heterosis
BR	Black rot
BSA	Bulked-segregant analysis

BSA-seq	Bulked-segregant analysis sequencing
BSR-seq	Bulked-segregant RNA sequencing
bZIP	Basic leucine zipper
CaM	Calmodulin
CaMV	Cauliflower mosaic virus
CAPS	Cleaved amplified polymorphic sequence
Cas9	CRISPR-associated 9 protein
CCA1	CIRCADIAN CLOCK ASSOCIATED 1
CC-NB-LRR	Coiled-coil NB LRR
CDF1	CYCLING DOF FACTOR1
cDNA	Complementary DNA
CDPK	Calcium-dependent protein kinase
CGMS	Cytoplasmic genic male sterility
ChIP	Chromatin immunoprecipitation
ChIP-seq	Chromatin immunoprecipitation sequencing
CI	Cytoplasmic inclusion
circRNA	Circular RNA
CK	Cytoplasmic kinase
cM	CentiMorgan
CMT	CHROMOMETHYLASE
CMV	Cucumber mosaic virus
CO	Constans
CoIP	Co-immunoprecipitation
Col	Columbia-0
COLDAIR	COLD ASSISTED INTRONIC NONCODING RNA
COLDWRAP	COLD OF WINTER-INDUCED NONCODING RNA FROM THE PROMOTER
COOLAIR	COLD INDUCED LONG ANTISENSE INTRAGENIC RNA
CP	Coat protein
CPB	Colorado potato beetle
CPGTH	Carboxypropyl glutathione
CR	Clubroot
CR	Cold responsive
CRISPR	Clustered regularly interspaced short palindromic repeats
CRTISO	Carotene cis-trans isomerase gene
CS	Chilling stress
CS	Climate smart
CWR	Crop-wild relative
CYP97A3	Carotene hydroxylase gene
DArT	Diversity arrays technology
DAS	Days after sowing
Dc	*Daucus carota* or carrot
DcAREB3	Carrot transcription factor to ABA-responsive elements
DcHSP	Carrot heat-shock protein

DCL	DICER-LIKE
DcPSY2	Carrot phytoene synthase2 protein (*gene*)
DDM1	Decrease in DNA methylation 1
DEG	Differentially expressed gene
DFR	Dihydroflavonol 4-reductase
DH	Doubled haploid
dpi	Days post inoculation
DREB	Dehydration responsive element binding protein
DRM	DOMAINS REARRANGED METHYLTRANSFERASE
E(z)	Enhancer of zeste
EBN	Endosperm balance number
ECD	European clubroot differential
eIF4E	Eukaryotic initiation factor 4E
EMS	Ethyl methanesulphonate
EpiRAD-seq	Epi-restriction site associated DNA sequencing
epiRILs	Epigenetic recombinant inbred lines
ER	Endoplasmic reticulum
ERF	Ethylene-responsive element binding factor
EST	Expressed sequence tag
ET	Ethylene
ET	Evapotranspiration
ETI	Effector triggered immunity
F_1	First filial generation
F3′,5′H	Flavonoid 3′,5′-hydroxylase
FAO	Food & Agriculture Organization (of the United Nation)
FAOSTAT	FAO statistics
FD	FLOWERING LOCUS D
FDA	Food and Drug Administration (USA)
FISH	Fluorescent *in situ* hybridization
FKF1	FLAVIN KELCH F BOX 1
FLC	FLOWERING LOCUS C
FLS	Flavonol synthase
Foc	*Fusarium oxysporum* f.sp. *conglutinans*
FOC	*Fusarium oxysporum* f.sp. *cepae*
FRI	FRIGIDA
Fru-2,6-P_2	Fructose 2,6-bisphosphate
FT	FLOWERING LOCUS T
FUL	FRUITFUL
FW	Fusarium wilt
G × E	Genotype × environment
GA	Gibberellin
gbM	Gene-body methylation
GBS	Genotyping-by-sequencing
GC-MS/MS	Gas chromatography-mass spectrometry

GD	Genetic distance
GEBV	Genome-estimated breeding value
GGT	γ-Glutamyl transpeptidases
GI	Gigantea
GIS	Geographic information system
Gly	Glycine
GMO	Genetically modified organism
GMS	Genic male sterility
GO	Gene ontology
GP	Genomic prediction
GPF	Green fluorescent protein
GRSV	Groundnut ringspot virus
GS	Genomic selection
GSPP	Good Seed and Plant Practices
GWAS	Genomewide association study
G×E×M	Genotype × environment × management
H_2O_2	Hydrogen peroxide
H3K27me3	Tri-methylation of the 27th lysine of histone H3
H3K36me3	Tri-methylation of the 36th lysine of histone H3
H3K4me3	Tri-methylation of the 4th lysine of histone H3
H3K9me2	Di-methylation of the 9th lysine of histone H3
HDR	Homologous recombination
HIB	High-efficiency integrated breeding
HIGS	Host-induced gene silencing
HP	High parent
HRM	High-resolution melting
HSF	Heat-stress transcription factor
HSP	Heat-shock protein
HT	High temperature
HVR	Hyper variable region
InDel	Insertion/deletion
IPCC	Intergovernmental Panel on Climate Change
IPT	Isopentytransperase
IRR	Interspersed repeat region
ISSR	Inter-simple sequence repeat
JA	Jasmonic acid
KASP	Kompetitive allele-specific polymerase chain reaction
KEGG	Kyoto Encyclopedia of Genes and Genomes
KYP	KRYPTONITE
LC-MS/MS	Liquid chromatography-mass spectrometry
LC-QqQ-MS	Liquid chromatography quadruple-mass spectrometer
LD	Long day
LD	Linkage disequilibrium
LEA	Late embryogenesis abundant
LF	Least fractionated

LG	Linkage group
LHP1	LIKE HETEROCHROMATIN PROTEIN 1
LHY	LATE ELONGATED HYPOCOTYL
lncRNAs	Long noncoding RNAs
LOD	Logarithm of odds
LP	Low parent
LRR	Leucine-rich repeat
MABC	Marker-assisted backcrossing
MAGIC	Multiparent advanced generation intercross
MAMP	Microbe-associated molecular pattern
MAPK	Mitogen-activated protein kinase
MAS	Marker-assisted selection
MBD-seq	Methyl-CpG-binding domain sequencing
mC	Methylated cytosine
MDB	Molecular design breeding
MeDIP-seq	Methylated DNA immunoprecipitation sequencing
MET1	METHYLTRANSFERASE I
MethylRAD	Methylation-dependent restriction site associated DNA
MF	More fractionated subgenomes
MIP	Major intrinsic protein
miRNA	Micro-RNA
MLMM	Multi-locus mixed model
MLPK	M-locus protein kinase
MPH	Mid-parent heterosis
MPV	Mid-parent value
mRNA	Messenger-RNA
MTMM	Multi-trait mixed model
MYB	Myeloblastosis oncogene
MYBR	Myeloblastosis oncogene responsive
MYC	Myelocytomatosis oncogene
MYCR	Myelocytomatosis oncogene responsive
NB	Nuclear-binding
NB-LRR	Nucleotide-binding leucine-rich repeat
NBS	Nucleotide-binding site
ncRNA	Noncoding RNA
NGS	Next-generation sequencing
NHEJ	Nonhomologous end joining
NILs	Near-isogenic lines
NIP	Nodulin-26 like intrinsic protein
NMR	Nuclear magnetic resonance
NRPD1	Nuclear RNA polymerase D1A
NRPE1	Nuclear RNA polymerase D1B
NUE	Nutrient use efficiency
OP	Open-pollinated
ORF	Open reading frame

OST2	OPEN STOMATA 2 gene
P5CS	Pyrroline-5-carboxylate synthetase
PAM	Protospacer adjacent motif
PAMP	Pathogen-associated molecular pattern
PAR	Photosynthetic active radiation
PAT	Phosphinothricin acetyltransferase
Pb	*Plasmodiophora brassicae*
PcG	Polycomb group
PCR	Polymerase chain reaction
PepMV	Pepino mosaic virus
PHD	Plant homeodomain
PIP	Plasma membrane intrinsic protein
Pol IV	Polymerase IV
Pol V	Polymerase V
PP2C-A	Protein phosphatase type 2C
PPR	Pentatricopeptide repeat
PR	Pathogenesis-related
PRC2	POLYCOMB REPRESSIVE COMPLEX 2
PRR	Pattern recognition receptor
PSII	Photosystem II
PTI	PAMPs/MAMPs triggered immunity
qPCR	quantitative PCR
QRL	Quantitative resistance loci
QTL	Quantitative trait locus
QTLs	Quantitative trait loci
R	Resistance
RAD-seq	Restriction site associated DNA sequencing
RAPD	Random amplified polymorphic DNA
RCA	Root cortical aerenchyma
RdDM	RNA-directed DNA methylation
RDR	RNA-DEPENDENT RNA POLYMERASE
retr	Recessive turnip mosaic virus resistance
Rf	Restorer-of-fertility gene
RFLP	Restriction fragment length polymorphism
RFO	RESISTANCE TO FUSARIUM OXYSPORUM
RGR	Relative growth rate
RILs	Recombinant inbred lines
RLCK	Receptor-like cytoplasmic kinase
RLK	Receptor-like kinase
RLP	Receptor-like protein
RNAi	RNA-interference
RNA-Seq	Ribonucleic acid sequencing
rnt1	Resistance and necrosis to tumv 1
ROS	Reactive oxygen species
rRF	Ribosomal RNA fragment

RSA	Root system architecture
RT	Reverse transcription
SA	Salicylic acid
SAM	Shoot apical meristem
SC	Self-compatibility
SCAR	Sequence-characterized amplified region
SCR	*S*-locus cysteine rich
SD	Short day
SE	Standard error
SET	SU(VAR)3-9, E(z), TRX
SI	Self-incompatibility
SIP	Small basic intrinsic protein
siRNAs	Small interfering RNAs
SIX1	Secreted-in-xylem 1
SLG	*S*-locus glycoprotein
Smi	*SP11*-methylation inducer
SMI	*SP11*-methylation-inducing region
SMRT	Single molecule real-time
snoRF	snoRNA fragment
SNP	Single nucleotide polymorphism
snRF	Small nuclear RNA fragment
SOC1	Suppressor of Overexpression of CO 1
SOD	Super oxidase dismutase
SP11	*S*-locus protein 11
SRAP	Sequence-related amplified polymorphism
SRK	*S* receptor kinase
SS	Salinity stress
SSH	Suppression subtractive hybridization
SSR	Simple sequence repeat
STF	*S*-locus retrotransposon family
STS	Sequence tagged site
SU(VAR)3-9	SUPRESSOR OF VARIEGATION 3-9
SUVH4	SU(VAR)3-9 HOMOLOG
SWI2/SNF2	Switch 2/sucrose non-fermentable 2
TALEN	Transcription activator like effector nuclease
TCSV	Tomato chlorotic spot virus
TDB	Transcriptome database
TE	Transposable element
TF	Transcription factor
TGRC	Tomato Genetic Resources Center (UC-Davis, USA)
TILLING	Targeting-induced local lesions in genomes
TIP	Tonoplast intrinsic protein
TIR-NB-LRR	Toll interleukin-1 receptor-NB-LRR
TLP	Thaumatin-like protein
TMV	Tobacco mosaic virus

ToBRFV	Tomato brown rugose fruit virus
ToMV	Tomato mosaic virus
tRF	tRNA fragment
tRNA	Transfer RNA
TRX	Trithorax
TSWV	Tomato spotted wild virus
TuMV	Turnip mosaic virus
TuRB01	*Turnip mosaic virus RESISTANCE IN BRASSICA 01*
TYLCV	Tomato yellow leaf curl virus
USDA	United States Department of Agriculture
VIN3	VERNALIZATION INSENSITIVE 3
VPg	Viral protein genome
VRE	Vernalization response element
WAKL22	WALL-ASSOCIATED KINASE-LIKE KINASE 22
WD	Water deficit
WGBS	Whole genome bisulfite sequencing
WGT	Whole genome triplication
WT	Wild type
WUE	Water-use efficiency
Xcc	*Xanthomonas campestris* pv.*campestris*
XIP	X intrinsic protein
Y2H	Yeast two hybrid
ZAT	Zinc finger of *Arabidopsis thaliana*
ZEP	Zeaxanthin epoxidase gene
ZF	Zinc finger
ZFN	Zinc finger nuclease
Zip	Zinc finger protein

Chapter 1
Climate-Smart Potato: An Integrated Breeding, Genomics, and Phenomics Approach

Jagesh Kumar Tiwari, Clarissa Challam, Swarup K. Chakrabarti and Sergio E. Feingold

Abstract Potato is an important source of food globally. Potatoes are among the most widely grown crop plants in the world, giving good yield under various soil and weather conditions. Yield losses of potato under current climate change keep increasing, despite the progressive increase in yield through breeding and management practices since the 1960s. Conventional breeding facilitated the development of high-quality potato with enhanced tolerance to severe environmental fluctuations such as drought, flooding, heat, and salinity. However, conventional approaches need to be complemented with advanced techniques in order to meet the increasing demands of the growing world population. The advances in marker-assisted and genomics-assisted breeding, sequencing technologies, and phenomics tools have enabled the potato improvement at a faster pace. The genomic resources have enabled the development of molecular markers associated with many important quantitative trait loci. It has also provided a clear picture of genomic variations in potato germplasm, and identified key genes for genetic engineering including genome editing. This knowledge is being utilized to facilitate the development of climate-smart potato. In this chapter, we discuss and summarize the advances in potato improvement through conventional and genomics-assisted breeding, genetic engineering, and phenomics approaches. This information could facilitate the incorporation of climate-smart traits (biotic and abiotic stresses) in modern breeding for more stable potato production with the changing climate.

Keywords Breeding · Climate change · Genomics · Phenomics · Potato

J. K. Tiwari (✉) · S. K. Chakrabarti
ICAR-Central Potato Research Institute, Shimla, Himachal Pradesh 171001, India
e-mail: jageshtiwari@gmail.com

C. Challam
ICAR-Central Potato Research Institute, Regional Station, Shillong, Meghalaya 793009, India

S. E. Feingold
Laboratorio de Agrobiotecnología EEA Balcarce, Instituto Nacional de Tecnología Agropecuaria (INTA), Balcarce, Argentina

C. Kole (ed.), *Genomic Designing of Climate-Smart Vegetable Crops*,
https://doi.org/10.1007/978-3-319-97415-6_1

1.1 Introduction

In recent years, the rapidly changing climatic conditions are hitting agriculture hard and are likely to increase the problems of food insecurity, hunger, and malnutrition for millions of people, particularly in South Asia, Sub-Saharan Africa, and small islands (Intergovernmental Panel on Climate Change, IPCC 2007). Global warming is causing changes in temperature at a rate unmatched by any temperature change over the last 50 million years. As shown in the IPCC (2007) report, the main repercussions of climate change are a rise in temperature, an increase in CO_2 concentration in the air, and an altered precipitation pattern. Among the changes, the increasing temperature has the most likely negative impact on the yield of crops including potato.

Potato is a global food security crop and is the fourth most important food crop after rice, wheat, and maize (Chakrabarti et al. 2017). Recently, Raymundo et al. (2018) evaluated the SUBSTOR-Potato model in various potato growing regions and concluded that there could be a global reduction in tuber yield from −2 to −6% by 2055, with a potential higher decline by 2085 (−2 to −26%). Similarly, climate change scenario is supposed to adversely affect potato production and productivity in India. Potato cultivation in India has largely been uneven as nearly 85% of potato in the country is produced in north Indian plains. The potato season (September–February) in this region is likely to be a little warmer also slightly drier with an increase in temperature ranging from 0.78 to 1.18 °C and corresponding precipitation decrease of 1−3%, by 2020 (Singh et al. 2013). The 1 °C rise in temperature associated with 400 ppm of CO_2 in the year 2020 (IPCC 2007) will result in a decline in potato production by 3.16%, without adaptation (Dua et al. 2013). The situation is expected to further worsen by the year 2050, where the atmospheric CO_2 concentration will be 550 ppm with a likely increase in temperature of 3 °C (IPCC 2007). Under this scenario, potato production is expected to fall by 13.72%, in the absence of needed steps (Singh et al. 2013; Anonymous 2015).

The world's population is widely expected to increase to at least 9 billion by 2050 (FAO 2013). This represents an increase of 2 billion people over the next 40 years, which will require a 70% increase in food production (Anonymous 2015). Potato being the fourth most consumed food crop species, there is a significant demand for crop improvements (Chakrabarti et al. 2017). Although the progressive increase in yield through breeding and management practices has been achieved in potato crop, the yield losses under current climate change keep increasing. Furthermore, climate change has a potential impact on the spread and severity of diseases caused by viruses, bacteria, fungi, and oomycetes (Castillo and Plata 2016; Lehsten et al. 2017). Therefore, accelerating the rate of genetic gain to adapt to climate change effects to meet the target demands of food production requires the integration of multidisciplinary research platforms/disciplines (Tester and Langridge 2010). This means there is a need to focus on key adaptive traits in order to maintain and increase crop productivity in increasingly unpredictable climate change. Applications of potato improvement through conventional and genomics-assisted breeding,

genetic engineering approaches, and available bioinformatics tools for potato are being discussed.

1.2 Prioritizing Climate-Smart Traits

Potato, (*Solanum tuberosum* Group Tuberosum L.) ($2n = 4x = 48$), represents one such heterozygous, polyploid crop that is clonally propagated by tubers (Potato Genome Sequencing Consortium 2011). While conventional breeding and genetic analysis are challenging in cultivated potato due to the abovementioned features, the majority of diploid potatoes possess gametophytic self-incompatibility. Historically, conventional breeding has been used to create improved potato cultivars. Yet due to its unique challenges, breeding is inefficient when complex traits need to be combined or if novel traits are not present in the desired germplasm. The key will be the combination of classical plant breeding with the advances in genomics, crop physiology, and modeling in an integrated profile involving genotype, phenotype, and environment.

1.2.1 Flowering Time and Tuberization

Flowering time is a key adaptive trait, responding to environmental and endogenous signals that switch between the vegetative and reproductive, while tuberization is the process of tuber formation from an underground stem called a stolon. Flowering and tuberization are distinct reproductive strategies in potato, both of which involve the sensing of the photoperiod by expanded leaf and generation of a signal in the leaves (a process referred to as induction), the subsequent transport of the signal (known as florigen or tuberigen), and the response in a distinct organ, the vegetative meristem or stolon tips (called as evocation). The genetic control of flowering time has been extensively studied in model species, particularly in Arabidopsis as well as in a number of important fields and tree crop species. However, the controlling factors involved in the tuberization process are not precisely clear and are under considerable investigation in recent decades (review by Dutt et al. 2017).

1.2.1.1 Plant Hormone Controlling Tuberization

Numerous studies have implicated the growth regulators as both inhibitor and promoter working coordinatedly to control tuber induction. The relevant literature has been reviewed from time to time. Gibberellins (GAs) have been implicated in different aspects of potato tuber formation. Several workers have shown that the non-induced state in potato plants is correlated with high endogenous GA levels. GA levels in the leaf decrease under short-day photoperiods and increase under long-day

conditions. *StGA2ox1* was found to be upregulated during the early stages of potato tuber development prior to visible swelling and was predominantly expressed in the subapical region of the stolon and growing tuber. In addition to GAs, several other plant hormones such as auxin, cytokinin, and ABA have been studied for their effect on tuber initiation. The initiation and induction of tubers in potato appear to be regulated by a cross talk between GA and auxin. Microarray experiments revealed a large number of auxin-related genes differentially expressed during early events in tuber development (Kloosterman et al. 2005). Examples of such genes are two PIN-like genes, an *adr11-2* (auxin downregulated) and an *acrA*-like (auxin-regulated gene containing a GTP-binding site) genes.

1.2.1.2 Day-Length Control of Flowering Time

Photoperiod sensing by the function of photoreceptors and the circadian clock appears to regulate flowering time via Arabidopsis CONSTANS (*AtCO*), a putative transition factor that accelerates flowering in response to long days (LDs). Mutations in the GIGANTEA (*gi*), CONSTANS (CO), and flowering locus T (FT) genes cause late flowering in LDs but do not affect flowering in short days (SDs), indicating a role of these genes in the LD flowering pathway. CO expression is reduced in the *gi* mutants, and overexpression of *AtCO* overcomes the late-flowering phenotype of these mutants. This transcription factor functions as an output to the clock and directly activates expression of the downstream floral regulator genes FT and Suppressor of Overexpression of CO 1 (*SOC1*, also known as *AGL20*). When the plant is exposed to light at this particular phase, flowering is induced in LD plants or delayed in SD plants.

The genetic factors controlling plant photoperiodic responses other than flowering are little known. However, interspecific grafting experiments demonstrated that the flower-inducing (florigen) tuber-inducing (tuberigen) signals are functionally exchangeable. Constitutive overexpression in potato of the Arabidopsis flowering-time gene *AtCO* impairs tuberization under short-day inductive conditions; *AtCO* overexpressing lines require prolonged exposure to short days to form tubers. Grafting experiments using these lines indicated that *AtCO* exerts its inhibitory effect on tuber formation by acting in the leaves. This module would involve the action of CONSTANS in the production of the elusive and long-distance acting florigen–tuberigen signal(s).

1.2.1.3 CONSTANS-Tuberization Control

Evidence for a role of the CO protein in daylength control of tuberization was also obtained in transgenic andigena plants expressing the *CO* gene from Arabidopsis. Three *CO* homologs also have been identified in potato, and evidence for a role in tuberization control has been obtained for one of these genes, designated *StCOL3*. *StCOL3* is cyclically expressed with a biphasic peak of expression at the end of the

night. Under SDs, *StCOL3* expression rises during the second half of the night and is still high during the first day hours (Martınez-Garcia et al. 2002). In LDs, the peak is narrower and occurs only during the day. Hence, this transcript peaks at a different time of the day than observed for the CO/Hd1 transcripts in Arabidopsis or rice. Despite such a difference in the timing of expression, *StCOL3* accumulation seems to fit with a similar model as that described in rice, and tuberization is promoted when *StCOL3* is expressed during the night but delayed when the expression of this protein coincides with light. Therefore, it will be interesting to compare the orthologs from potato, rice, or the SD plant *Pharbitis nil* with the CO Arabidopsis protein, and to search for conserved domains that might explain the differential regulatory function of the SD proteins (Martınez-Garcia et al. 2002).

1.2.1.4 Transcription Factors

MADS-box genes are an example of a family of highly conserved transcription factors (TFs) that have diverse roles during plant development. In the early flowers, POTM1-1 transcripts were accumulated abundantly in the developing reproductive organs including the placentae of carpels and the pollen sacs of stamens. In contrast, the pattern of POTM1-1 distribution during late flower development was different from that of early flower development. The POTM1-1 transcripts were abundant in the sepals and petals of late flowers but were minimally expressed in the stamens and carpel. In the shoot apical meristem of the vegetative organs, transcripts were distributed throughout meristem domes, young leaves, and developing vascular cambium (Kloosterman et al. 2013). In the early tuberization, the transcripts were widely distributed in the swollen tips of the stolons. Taken together, the results suggested that POTM1–1 gene expression was temporally and spatially regulated in actively growing tissues of both vegetative and floral organs with specific distribution patterns dependent upon the developmental stages of the tissue. In another study, TFs family genes *ABF4* and *ABF2* transgenic potato exhibit ABA hypersensitivity during tuberization, accompanied by a GA deficient phenotype. *ABF4* expression triggered a significant rise in ABA levels in stolons under tuber-inducing conditions as compared with wild-type plants and transcriptional deregulation of GA metabolism genes. These results demonstrated that Arabidopsis *ABF4* functions in potato ABA–GA signaling cross talk during tuberization by regulating the expression of ABA and GA-metabolism genes. Hendriks et al. (1991) have reported that patatin and four serine proteinase inhibitor genes are differentially expressed during potato tuber development. The studies showed that the length of the day/light conditions differently influenced the expression level of these individual genes.

1.2.1.5 Molecular Targets for Tuberization

StSP6A (FT-like; Arabidopsis ortholog) is a mobile signal that has been shown to positively regulate tuberization transition in potato. Recently, it has been reported

that both photoperiod dependence on tuberization and the duration of the potato growing cycle are linked to a regulatory gene called *StCDF1* (Kloosterman et al. 2013). *StCDF1* acts as an intermediary in the way of signaling between the circadian clock mediated by the *Gi* (GIGANTEA) gene and the photoreceptors of blue light and *StSP6A* (Navarro et al. 2011; Abelenda et al. 2014). Natural allelic variants of the *StCDF1* gene could be responsible for the adaptation of potato at high latitudes, generating the Tuberosum group. Another FT member of potato, *StTFL1* has been suggested to increase the number of tubers produced when overexpressed. Two proteins, *StBEL5* and *POTH1* (transcription factors belonging to TALE superclass), have been proven to be positive regulators of the tuberization process in potato and can also be prominent candidates for improving tuberization through their simultaneous overexpression (Dutt et al. 2017). Other genes/proteins that are suggested for genetic engineering through overexpression include *POTM1*, *StPA2Ac, StTUB19, StTUB7, StABF2,* and *StABF4*. Whereas, *StCO TF*, *StSP5G,* and *StSUT4* sucrose transporters have been found to inhibit tuberization. Hence, their suppression may be utilized for promoting tuberization.

1.2.2 Cold Tolerance

Among the different abiotic stresses, cold is an essential factor that limits crop productivity worldwide. Low temperature affects the growth and development of agronomic species throughout the world. It is very important to study the frost damage mechanism and to breed cold-tolerant varieties since the average minimum temperature is below 0 °C in about 64% of the earth's land area and it is below −10 °C in about 48%. Potato crop adaptation is needed to increase production and stability under cold conditions that are getting worse with climatic change. Plants have adapted two mechanisms to protect themselves from damage due to below freezing temperatures. First, supercooling is a low-temperature tolerance mechanism that is usually associated with acclimated xylem parenchyma cells of moderately hardy woody plants. The second and most common low-temperature response mechanism is acclimation. Acclimation is a gradual process during which there are changes in just about every measurable morphological, physiological, and biochemical characterization of the plant (Takahashi et al. 2013). These changes are determined by genotype and environmental interactions that are quite complex.

1.2.2.1 Genetic Variation in Cold Tolerance

Many primitive cultivars and wild relatives of potato can tolerate environmental stress conditions in their habitats. Frost tolerance may be one of the oldest objectives of potato breeding. A very old study showed frost resistance or tolerance using hybrids between *S. demissum* and other susceptible species. Frost tolerance also occurred in certain accessions of *S. commersonii* and its hybrids. Bukasov (1933) evaluated

the frost resistance of several wild potato species and hybrids in the winters of the years 1930–31 and 1931–32. *S. demissum, S. acaule*, and *S. juzepczukii* were not affected by frost of −6 °C, *S. demissum* and *S. ajanhuiri* showed different reactions in different plants, and *S. andigenum* perished entirely under the same conditions, with the exception of one variety "Pacus," which proved to be resistant.

1.2.2.2 Gene Expression in Response to Cold Tolerance

Extensive researches have been conducted to improve the understanding of the biochemical and molecular basis of the cold acclimation response and the changes that take place throughout this process. However, the increase in cold tolerance obtained by acclimation is not static. Extensive physiological and biological changes occur during cold acclimation starting with a reduction in the growth rate and water content of various plant tissues. Through the cold acclimation process reprogramming of gene expression and various modifications in the metabolism take place (Chinnusamy et al. 2010). Acclimation also causes an increase in the production of antioxidants, abscisic acid (ABA), and compatible osmolytes such as soluble sugars and proline. A number of cold-responsive genes have been reported in various plant species: *COR* (cold-regulated) genes, *LEA* (late-embryogenesis abundant) genes, regulatory genes, antifreeze protein genes, and the genes encoding signal transduction proteins.

Proline has been shown to improve cold tolerance and aid cell structure protection in many crops, such as maize, potato, wheat, and barley, and in *L. perenne* had shown to improve osmotic adjustment during cold acclimation. Intracellular accumulation of endogenous polyamines (PA) occurs in response to cold stress as they contribute to plant response to low-temperature conditions. The increase in levels of diamine putrescine (Put) has been reported in cold-stressed Arabidopsis (Kaplan et al. 2004). The increased titers of Put on overexpression of S-adenosylmethionine decarboxylase (*StSAMDC)* were actually the result of high spermidine accumulation which was actively interconverted to Put by acetylation.

1.2.2.3 Role of CBF (C-Repeat Binding Factor) Gene

The *CBF* genes are the key regulatory elements in cold-responsive signaling pathways and hence serve as potential targets of genetic manipulation to engineer cold stress-tolerant plants. *CBFs* are discovered in all important field crops and some vegetable species like potato (Sanghera et al. 2011). Transgenic Arabidopsis plants overexpressing *CBF1* showed freezing tolerance while avoiding the negative impact of cold stress on development and growth characteristics. Constitutive overexpression of cold-inducible transcription factors like CBF1 has been shown to impart cold stress tolerance, through introduction of *CBF1* cDNA into chilling-sensitive tomato under the control of strong CaMV35S promoter (Hsieh et al. 2002). Another candidate target is the *CBF4*, a close *CBF/DREB1* homolog, whose overexpression alleviated both freezing and drought stress in Arabidopsis. Transgenic potato

and poplar plants expressing soybean cold-inducible C2H2-type zinc finger transcription factor (*SCOF-1*) increased cold and freezing stress tolerance in Arabidopsis. Overexpression of *bHLH* TFs with clone names such as *StMHJ91, StMEK79, StMDC31, StMDE79, StMDV67, StMER91, StMHZ85*, and *StMCU25* increase cold stress tolerant to potato.

1.2.2.4 Role of Ca^{2+} Signal Pathway

Ca^{2+} is considered to be the main signal transducer in signaling cascades motivated in response to plant abiotic stress types. Upon cold stress, cytosolic Ca^{2+} concentration immediately rises up to a level of designated Ca^{2+} signatures for cold. This designated cytoplasmic Ca^{2+} signature is decoded by Ca^{2+} sensors like Calmodulins (CaM), Calmodulin-like proteins (CMLs), Ca^{2+}-dependent protein kinases (CDPKs), Calcineurin B-like proteins (CBLs), and their interacting kinases (CIPKs) to transduce the signal intracellularly. Therefore, differentially expressed Ca-related genes in chilling-stressed potato could have major functions in intracellular signal transduction, thereby, in the development of cold acclimation. Moreover, reactive oxygen species (ROS) also play an important role as second messengers responding to various abiotic stresses. Some of the authors reported that abiotic stresses cause an oxidative burst and that a low level of ROS induces an increase in Ca^{2+} influx into the cytoplasm. The high level of Ca^{2+} activates NADPH oxidase in order to produce ROS through yielding O^{-2} which is then converted to H_2O_2 under the effect of super oxidase dismutase (SOD). Therefore, the production of ROS is Ca^{2+} dependent and the concentration of Ca^{2+} is also regulated by the concentration of ROS by the activation of Ca^{2+} channels in the plasma membrane. Therefore, a cross talk between Ca^{2+} and ROS modulates the activity of specific proteins that control the expression-specific definitive defense genes in the nucleus.

1.2.2.5 Role of Phytohormones

The existence of an ABRE cis-acting element (ABA-responsive element) is an essential requirement for the upregulation of ABA-induced gene expression (Shinozaki and Yamaguchi-Shinozaki 2000). Finkelstein et al. (2002) reported an important role of ABA in the induction of *LEA* gene expression. The role of ABA in the upregulation of *LEA* genes is considered to be one of the mechanisms that ABA has to increase plant drought and freezing tolerance. Moreover, the application of salicilic acid (SA) improved the cold tolerance of several plant species such as potato, rice, and maize. Gibberellin (GA) is the other plant hormone altered in plants under cold stress. It has been found that GA is involved in the expression of *CRT/DRE*-binding factor gene which in turn confers tolerance to drought, salt, and cold stress. Plant phytohormone jasmonic acid (JA) also plays an essential role as an important regulatory signal in plant cold tolerance. GA is associated with SA/JA balance in the CBF-mediated stress response. It has been proved that the external application of JA significantly

enhanced cold tolerance in plants with or without acclimation. Moreover, blocking of the endogenous JA increased the sensitivity to the cold stress. It has been proved that JA upregulated the *CBF/DREB1* signaling pathway (Hu et al. 2013).

1.2.3 Drought Tolerance

Most potato varieties have sparse and shallow root system and are vulnerable to a series of abiotic stresses, including drought and high salinity, thus resulting in a reduction in tuber yield and quality. Even short periods of drought stress can result in serious damage and cause a severe reduction in tuber production. Research on drought tolerance in potato only started during the period 60–80s as it was not considered as a major yield-limiting factor in potato for a long time. The situation drastically changed over the last few years due to the increasing importance of drought for potato production and the recognized interest in developing potato cultivars able to perform well in drought-prone areas. Moreover, in production areas under irrigation, drought tolerance and water use efficiency are of importance as there is a growing concern on carbon and water footprints. Similarly, a reduction of irrigation where water quality is poor will prevent salinity in soils enhancing sustainability. Knowledge of physiological mechanisms underlying drought tolerance in potato (e.g., the role of abscisic acid, osmotic adjustment, or rooting patterns) is however still poor compared with other crops.

1.2.3.1 Genetic Variation in Drought Tolerance

Screening for drought tolerance in potato landraces has been performed by many researchers. A high proportion of accessions combining drought tolerance with high irrigated yield was found in Andean landraces, particularly in the species *S. curtilobum* (Juz. and Bukasov) in the *S. tuberosum* L. cultivar groups Stenotomum, Andigenum, and Chaucha. Watanabe et al. (2011) identified *S. chillonanum, S. jamesii,* and S. *okadae* as potential drought-tolerant species by screening 44 accessions of wild species selected based on their drought habitats derived from geographic information system (GIS).

1.2.3.2 Root System Architectures (RSA)

Root systems are usually involved in both drought avoidance and tolerance during water deficits due to the constitutive and plastic characteristics of roots. RSA is also highly plastic to respond rapidly to environmental changes such as water deficit. Liu et al. (2005) found that the concentration of ABA in the xylem of potato plants increases significantly as the substrate contains less water. This suggests that the roots of potato plants are able to perceive the lack of water in the substrate and in response

to this situation produce ABA. When plants perceive water deficit stress, roots tend to keep growing and penetrate into deeper soil layers. The ability of plants to develop deeper rooting systems under drought stress depends on the tolerance levels of the roots to water deficit stress. In addition to deep rooting, drought stress also induces the plasticity responses of root systems by increasing the number of fibrous roots, decreasing lateral root diameter, and fluctuations in root biomass. Alterations in root anatomy, such as aerenchyma formation in maize, save the energy inputs to allow improved soil penetration and exploration to compensate water deficit (Wishart et al. 2013).

Breeding of new cultivars with excellent root characteristics to absorb water from deeper regions of the soil and under lower soil water potential will increase the usage of soil water and contribute to efficient utilization of water from precipitation or irrigation in potato production. Many studies found a positive relationship between the size of the root system and the amount of aboveground biomass. Quantitative trait locus (QTL) mapping has been conducted in potato and many QTLs associated with RSA and drought tolerance have been mapped. It can be concluded that the plants that have a more-developed root system at greater depths of the soil profile tend to have milder reactions to drought.

1.2.3.3 Water Use Efficiency (WUE)

Improving water use efficiency is another promising strategy to overcome drought stress. The essential factors to improve water use efficiency are to conserve water in plants and reduce the unnecessary transpiration losses. QTL analysis of near-isogenic lines of Arabidopsis has identified numerous QTLs involved with WUE, some co-localized with flowering-time QTLs involved with drought avoidance. However, some of these genes have been shown to be independent of QTL analyses, and it is possible to select for higher WUE while leaving out flowering-time QTLs. Molecular genetics represent an essential approach for identification and elucidation of the various traits that contribute to WUE. Some characterized genes have been identified that control water uptake and loss. To fully utilize knowledge of these genes to improve WUE, an integrated approach is required that implements functional characterization of promising QTLs, high-throughput phenotyping, field validation of traits, and stacking/pyramiding of these traits into WUE-efficient and drought-tolerant varieties for agriculture. This challenge represents one of the most complex tasks facing biotechnology today and will require both modern breeding and gene editing techniques to achieve. Regardless of the challenge, molecular genetics will be essential in the identification and characterization of genes that play an important role in increasing WUE and drought tolerance.

Molecular Strategies for improving WUE

Advances in genetics, "omics," precise phenotyping, and physiology coupled with new developments in bioinformatics and phenomics are or will be providing means for dissecting integrative traits that affect adaptation to stressful environments. In

this regard, it has been indicated that analyzing the effect of traits on crop yield with the aid of modeling and confirming through field experiment (and sound biometrics) will lead to identifying favorable alleles for enhancing adaptation to a stress-prone environment. Some traits used as proxy for selecting germplasm with enhanced adaptation to drought-prone environments (especially among grain crops) are anthesis–silk interval, early flowering (that could provide partial relief to water shortage during grain filling), floral fertility (by minimizing severe water deficit-induced damage at flowering), early vigorous growth (which improves crop establishment and reduces soil evaporation), root architecture and size (for optimizing water and nutrient harvest), and tiller inhibition (that increases tiller survival rates and carbohydrate storage in stems for ensuring further grain filling), among others (Tuberosa et al. 2007). Likewise, indirect selection has been used for improving WUE, e.g., through canopy temperature depression, carbon isotope discrimination (Δ13C) for C_3 crops (although both may differ across locations), and ear photosynthesis (Tambussi et al. 2007). Recent molecular approaches offer new alternatives to improve drought tolerance in several plant species, including potato, in terms of the identification of signaling pathways and master genes regulating drought tolerance. For example, hypersensitivity to ABA has been associated with a better behavior under water stress (Papp et al. 2004). Among the components involved in the transduction of the ABA signal, genes encoding phosphatases, protein kinases, and transcription factors have been identified (Xie et al. 2010; Christmann et al. 2006). Genomic tools for identifying genome regions and genes involved in the control of drought tolerance should be more extensively used in potato. More detailed information will become available in the future using the metabolomics and proteomics techniques together with integrated bioinformatics systems. These advances will facilitate the genetic engineering of single or multiple targets to create a cultivated phenotype with high-yielding potential under drought stress conditions. Changes in the gene expression profiles are induced in response to drought stress and several genes are regulated up or down with osmotic stress.

1.2.4 Heat Tolerance

Heat stress affects growth, quality, and yield traits by impacting the structure and metabolic functions of cells and several physiological processes, such as structural alterations of protein complexes, changes in protein synthesis and enzyme activities, cellular structure and membrane functions, production of detrimental reactive oxygen species, decoupling of metabolic pathways, and damage to the photosynthetic apparatus. The ideal temperature for potato aerial growth is 20–25 °C and the optimum temperature for tuber formation in 15–20 °C (Rykaczewska 2013). In fact, higher temperatures adversely affect tuber formation and tuber development in potato, and this inhibition of tuberization has been linked to the inhibition of tuberization signal StSP6A (an ortholog of Arabidopsis flowering *FT* locus) at elevated temperatures (Hancock et al. 2014) and reduced accumulation of carbon into starch in

the tuber at higher temperatures. Also, an adverse effect on photosynthesis resulting from chlorophyll loss and reduced CO_2 fixation has been reported for tuber-forming Solanum species.

A large number of differentially expressed genes involved in many biological processes and molecular functions as well as differential metabolite accumulation have been identified in response to mild to moderate heat stress in potato leaves and tubers. Tolerance to elevated temperatures in potato is likely a polygenic trait and, thus expected to be substantially influenced by genotype-environment interactions. As such, potato cultivars may show a wide variety of variations in their response to heat stress. However, so far most studies on heat stress response of potato have focused on some germplasm accessions (Reynolds and Ewing 1989) or only on a very few registered cultivars. In order to understand the biological basis of heat tolerance and select and develop potato varieties that are heat tolerant, it is critical to understand the variation in response of a large number of potato varieties/cultivars to heat stress. Indeed screening and breeding for heat-tolerant potato cultivars are urgently needed to stabilize potato productivity in the current and future warmer environment.

Maximum threshold temperatures at which high temperatures kill seedlings can depend on plant preconditioning. Seedlings subjected to high but sublethal temperatures for a few hours subsequently can survive higher temperatures than seedlings that have been maintained at moderate temperatures. This acclimation to heat can be induced by the gradual diurnal increases in temperature that occur in hot natural environments (Vierling 1991). The heat shock response involves repression of the synthesis of most normal proteins and mRNAs, and the initiation of transcription and translation of a small set of heat shock proteins (Vierling 1991). Studies of loss-of-function mutants of Arabidopsis thaliana demonstrated that the enhanced thermotolerance can be associated with at least three independent effects: the synthesis of a novel set of proteins (specifically Hsp101), protection of membrane integrity, and recovery of protein activity/synthesis (Queitsch et al. 2000). In order to combine multiple sources of heat tolerance, recurrent selection has been employed in diploid potato resulting in a 27% increase in yield in a single cycle of recurrent selection and is being employed to combine heat and drought tolerance in common bean.

Considered to be the most important environmental factor influencing the quality and yield of potato (Rykaczewska 2013), high temperature affects various biochemical and physiological processes in potato plants. High temperature negatively affects the tuber initiation and development by inhibiting the tuberization signal, StSP6A (Navarro et al. 2011). High temperature also causes nutrient source–sink problems by decreasing the carbon assimilation in tubers and inhibition of tuber filling (Krauss and Marschner 1984). Hence, high temperature, in turn, leads to reduced tuber quality and yield. Heat stress also causes a decrease in photosynthesis by decreasing the gas exchange and chlorophyll biosynthesis (Reynolds and Ewing 1989).

The heat stress causes osmotic and oxidative stresses in plants. Plants have evolved different defense mechanisms, such as avoidance and tolerance, activated under osmotic and oxidative stresses. Extended periods of drought or high temperatures lead to the production of reactive oxygen species, which are cytotoxic in high concentrations. Because reactive oxygen species are not only toxic but also participate

in signaling events, plant cells require at least two different mechanisms to regulate their intracellular reactive oxygen species concentrations by scavenging of reactive oxygen species: one that will enable the fine modulation of low levels for signaling purposes, and one that will enable the detoxification of excess reactive oxygen species, especially during stress (Mittler 2002). To date, several transcriptomic studies have been completed in potato development and abiotic/biotic stress response (Massa et al. 2013); however, the number of transcriptomic studies carried out to elucidate the changes in the gene expression profiles of potato under high temperature is limited. There are various studies exploring the expression patterns and functions of individual genes in potato under heat (Monneveux et al. 2014).

Improvement of potato heat tolerance has been moderately successful because there are limited numbers of studies on understanding the molecular mechanism of heat tolerance in potato (Levy and Veilleux 2007). In order to better understand the heat tolerance mechanisms of potato, the key genes and overall network of genes acting in the heat tolerance of potato should be characterized in more detail. To our knowledge, only a few studies are available on the heat response of potato at the molecular level. In one of the studies, 2190 genes were found to be differentially expressed in potato leaves when the plants were exposed to moderately elevated temperatures (30/20 °C, day/night) for up to 5 weeks (Hancock et al. 2014). Heat-responsive genes involved in photosynthesis, lipid metabolism, and amino acid biosynthesis were highly overrepresented at all time points of stress treatment. In tubers, a total of 2886 genes exhibited major changes in their transcript levels associated with the different temperature conditions in the course of stress treatment. Differentially expressed genes in potato tubers were underrepresented in functional categories related to cell wall processes, lipid metabolism, aspects of secondary metabolism, hormone metabolism, biotic stress, DNA metabolism, and development, whereas genes involved in RNA metabolism were overrepresented following moderately high-temperature treatment. In k-means clustering of heat-responsive transcripts of potato, genes associated with ABA, ethylene, auxin, and brassinosteroid responses; heat shock proteins and transcription factors; and genes previously associated with abiotic stress responses were identified. These data indicate that the potato plants respond to moderately elevated temperatures differently than other crops such that instead of known symptoms of abiotic stress, they exhibit a combination of different biochemical and molecular pathways during tuber development. The number of transgenic studies to improve the high-temperature tolerance in potato is limited.

1.2.5 Salinity Tolerance

Potato leaves are very sensitive to saline water and are severely damaged by overhead irrigation with saline water. Uptake of chlorine and sodium by leaves may induce toxicity, exhibited as leaf burn along the margins. Fidalgo et al. (2004) reported that salt stress negatively affected relative water content, leaf stomata/conductance, and

transpiration rate of the cultivar Desiree. Changes to the chloroplast structure presumably affect photosynthesis, resulting in increased starch in leaves, suppression of nitrate reductase activity and reduced growth and dry matter production in tubers (Ghosh et al. 2001). Saline water increases the proportion of exchangeable sodium ions in the soil solution, leading to formation of sodium carbonate, which raises the pH. These alkaline conditions reduce the availability of nutrients, such as phosphate, iron, zinc, and manganese, to the plants. In soils rich with calcium carbonate, this damaging process is inhibited, a phenomenon that has been reflected in vitro where supplemental calcium alleviated salinity-induced nuclear degradation in root meristematic cells (Richardson et al. 2001). Abdullah and Ahmad (1982) found that the addition of 2% gypsum to saline soil improved the yield of potatoes grown in pots, increased their protein, potassium and calcium content, and decreased the level of glycoalkaloids.

1.2.5.1 Genetic Variation for Salinity Stress Tolerance

Differences in the response of wild potato species and potato cultivars to salinity have been reported. *S. kurzianum* was also found to be tolerant to salt in greenhouse trials on whole plants, but its callus was no more resistant than cultivar controls (Sabbah and Tal 1995). *S. juzepczuckii* and *S. curtilobum* were also identified as salt-tolerant by their ability to form microtubers in medium with added NaCl, whereas microtuber production from *S. tuberosum* declined markedly with increasing salt concentrations (Silva et al. 2001). In a study of four potato cultivars irrigated with water at four different salinity levels, Elkhatib et al. (2004) identified cv. Cara as the most tolerant to salinity. Different methods have been proposed to mitigate the negative effect of salinity on potato. Application of proline spray on potato leaves alleviated the adverse effects of salt stress on potato growth by maintaining ion and water availability and protecting potato photosynthesis against salt-induced oxidative stress. These results suggested that foliar application of nutrients could be used to improve potato tolerance to salinity by alleviating the adverse effects of salinity on growth and reproductive yield. Calcium is believed to play an important role in stress tolerance and may be responsible for the observation of salt-tolerance QTLs.

1.2.5.2 In Vitro Screening for Salinity Stress Tolerance

The in vitro system was considered adequate for screening for salt tolerance and was used to demonstrate that exogenously supplied proline provided a measure of protection against salt stress (Prasad and Potluri 1996). Using an in vitro screen for salt tolerance, Rahnama and Ebrahimzadeh (2005) demonstrated differential activities of antioxidant enzymes between salt-tolerant and sensitive potato cultivars, suggesting that the tolerant cultivars may be better protected against reactive oxygen species by their ability to increase the activity of antioxidant enzymes under salt stress.

Zhang et al. (2005) observed differences in salt sensitivity between two potato cultivars using an in vitro microtuberization system. The effects of 5-aminolevulinic acid (ALA), a key precursor in the biosynthesis of porphyrins such as chlorophyll and heine, promoted development and growth of potato microtubers and enhanced protective functions against oxidative stresses (Zhang et al. 2006).

The salt tolerance of *S. juzepczuckii* and *S. curtilobum* was positively correlated with leaf proline content, suggesting that leaf proline accumulation could be used as a marker for salt tolerance in potato (Martinez et al. 1996). However, Velásquez et al. (2005) found no association between proline accumulation and salt tolerance among 12 Argentine Andean potatoes although considerable phenotypic variation was observed among these varieties in an in vitro screen. Likewise, Rahnama and Ebrahimzadeh (2005) found no clear relationship between the accumulation of proline and salt tolerance in potato seedlings. Effective in vitro selection for salt tolerance was similarly reported by Burgutin et al. (1996), who identified five of 38 somaclones that maintained superior performance in field tests over several years.

1.2.5.3 Molecular Tools to Address Salinity Stress Tolerance

Although breeding for tolerance to salinity in crop plants has had limited success, new technologies offer some promise. Ryu et al. (1995) identified nine proteins in *S. commersonii* that were induced by 24 h salt treatment; a subset of these same proteins was also induced by cold stress or ABA treatment. Two drought-induced stress proteins, *CDSP 32* and *CDSP 34*, which accumulate in the stroma and thylakoids, respectively, were found to be similarly induced by salt stress (Pruvot et al. 1996). However, only *CDSP 34* expression was enhanced by exogenous abscisic acid application, indicating different signaling pathways for the two proteins.

The availability of genome sequence information and tools for functional genomics has already been used to improve tolerance to salinity in transformed crop plants (Apse and Blumwald 2002). Zhang and Blumwald (2001) showed that transgenic tomato plants overexpressing a vascular Na^+/H^+ antiport from *Arabidopsis thaliana* were able to grow, flower, and fruit in the presence of 200 mM NaCl. Zhang et al. (2001) transformed *Brassica napus* with the same gene and observed a similar result. The introduction of a single alien gene has also been used to attempt to improve salt stress tolerance of potato. Celebi-Toprak et al. (2005) transformed *S. tuberosum* cv. Desiree with the DREBIA gene under the control of a stress-inducible promoter (rd29A) from *Arabidopsis*, and selected nine of 78 transformants that exhibited salinity tolerance. In contrast, transgenic lines of potato cv. Nicola that expressed a pyrroline-5-carboxylate synthetase cDNA from *Arabidopsis* exhibited both increased accumulation of proline under salt stress as well as a less severe reduction in tuber yield compared to non-transgenic controls.

1.2.6 Disease Resistance

1.2.6.1 Late Blight (LB)

Late blight (caused by *Phytophthora infestans*) is the most serious disease of potato worldwide. Studies conducted at International Potato Center, Lima, Peru, to work out the risk of late blight (expressed as number of sprays) at global-level climate change scenario revealed that with rise in global temperature of 2 °C, there will be lower risk of late blight in warmer areas (<22 °C) and higher risk in cooler areas (>13 °C). Earlier onset of warm temperatures could result in an early appearance of late blight disease in temperate regions with the potential for more severe epidemics and increased number of fungicide applications needed for its control. An increase in both, temperature and relative humidity, has added a new dimension to late blight across the world. In recent years, the development of durable and extreme resistance to LB disease, using resistance genes from several wild potato species collected from Central America and Andean South America, has been attempted.

Genetic Variation for Late Blight Resistance

Potentially more long-lasting, broad-spectrum *R* genes such as *RB*/*Rpi-blb1* (Song et al. 2003; van der Vossen et al. 2003), *Rpi-blb2* (van der Vossen et al. 2005), *Rpi-blb3* (Lokossou et al. 2009, Park et al. 2005) from *S. bulbocastanum*, *Rpi-sto1* from *S. stoloniferum*, *Rpi-pta1* from *S. papita* (Vleeshouwers et al. 2008), and *Rpi-vnt1.1* from *S. venturii* (Foster et al. 2009, Pel et al. 2009) have been identified and cloned. Additional *R* genes have been described in other potato wild relatives from *S. berthaultii* (Ewing et al. 2000; Rauscher et al. 2006), *S. capsicibaccatum* (Jacobs et al. 2010), *S. spegazzinii* × *S. chacoense* (Chakrabarti et al. 2014), *S. microdontum* (Tan et al. 2008), *S. mochiquense* (Smilde et al. 2005), *S. paucissectum* (Villamon et al. 2005), *S. phureja* (Śliwka et al. 2006), *S. pinnatisectum* (Kuhl et al. 2001), *S. ruizceballosii* (Śliwka et al. 2012), *S. schenckii* (Jacobs et al. 2010), *S. sparsipilum* and *S. spegazzinii* (Danan et al. 2009), *S. stoloniferum* (Wang et al. 2008), and *S. verrucosum* (Jacobs et al. 2010, Liu and Halterman 2006). Many R genes have been deployed from different sources through marker-assisted selection (MAS) and/or transgenic approach against late blight (review by Tiwari et al. 2013).

1.2.6.2 Soil-Borne Pathogens

The effect of climate change on soil-borne pathogens would vary from pathogen to pathogen. *Synchytrium endobioticum* causing wart and *Spongospora subterranea* responsible for powdery scab are favored by low temperature and high soil moisture. Wart spores, although can cause infection in the range of 10–28 °C with an optimum of 21 °C, there is hardly any infection beyond 23 °C. Therefore, warmer climates are likely to reduce wart infestation. Powdery scab infestation is also likely to be reduced with an increase in temperature and reduction in rainfall as a consequence of global warming. Diseases like *Sclerotium* wilt, charcoal rot, and bacterial wilt are favored by

high temperatures and moisture. Optimum temperature requirement for this disease is 30–35 °C. Similarly, bacterial wilt may also advance to higher altitudes in hilly regions due to global warming, making them unfit for seed production. Charcoal rot is currently endemic in the eastern parts of India. Global warming is likely to increase the severity of this disease in these regions. It is also likely to expand to other parts of North Central plains as well. Black scurf and common scab are favored by moderate temperatures (15–21 °C and 20–22 °C, respectively) and are likely to remain insulated from global warming in the near future. By the end of the century when ambient temperatures are likely to increase by 1.4–5.8 °C, the severity of these two diseases may decrease substantially.

Genetic Variation for Bacterial Wilt Resistance

Resistance to bacterial wilt has been found in *S. phureja* (Fock et al. 2000), *S. stenotomum* (Fock et al. 2001), *S. commersonii* (Kim-Lee et al. 2005), and *S. chacoense* (Chen et al. 2013). The resistance genes have been transferred to cultivated potato by protoplast fusion (Chen et al. 2013).

1.2.6.3 Viral Diseases

The rate of multiplication of most of the potato viruses gets increased with the increase in temperatures. In the subtropical plains, where the majority of the potatoes are grown, global warming may not affect potato viruses directly, but may have a serious repercussion through the altered biology of insect vectors. The increase in temperature will enhance the vector population, thereby increasing the number of insecticide sprays for keeping the vector population in check. The rate of multiplication of the virus in host tissue will also increase substantially, leading to early expression of the virus symptoms. Studies carried out in Holland revealed that during the last decades, some new viral strains ($PVYnt^{n}$ and PVY^{nw}) have been detected indicating that climate change may introduce new viral strains by viral genome recombination favored by a simultaneous infection in one plant.

As regards to insects, *Bemisia tabaci* was a minor pest until recently in India. Data on population buildup during the last 20 years revealed that the average population of *B. tabaci* was 11 whitefly/100 leaves during 1984 which rose to 24.24 in 2004. During this period, the average ambient temperature increased by 1.07 °C. This indicates that warming may lead to whitefly infestation in Indo-Gangetic plains. Increase in *B. tabaci* population has also led to the outbreak of a new viral disease known as Apical leaf curl in potato which has since been identified to be caused by a Gemini virus which was not previously reported to infect potato. Therefore, a new dimension has been added to seed potato production in subtropics. Results also tend to suggest that in subtropical plains of India, *Myzus persicae* population is on the rise. Besides, aphid appearance advanced by 5 days during the last 20 years, reducing the low aphid pressure window for seed production from 80 to 75 days. On the other hand, the population of *Aphis gossypii* has increased threefold during the last 20 years. Although *A. gossypii* is not an efficient vector, its appearance right from the emergence and further maintaining its population throughout the crop

season may pose serious problems to seed production in subtropical plains. Leaf hopper (*Empoasca fabae*) is another pest which has assumed significance in early planted crop in subtropical plains of India.

Resistance to Potato Virus Y

Potato virus Y (PVY), one of the most important diseases of potato, can reduce yield by 80% (Hane and Hamm 1999). The Ry_{adg} gene, conferring extreme resistance to all known PVY strains, has been mapped and cloned from *S. andigena* (Hamalainen et al. 1997). In mild winters, high intensity of aphid movement during spring and a high frequency of PVY-infected potatoes have been reported. Aphid vectors are expected to have increased survival with milder winter temperatures, and higher spring and summer temperatures will increase their development and reproductive rates and lead to more severe disease. Kasai et al. (2000) developed sequence-characterized amplified region (SCAR) markers to detect PVY resistance of the gene *Ryadg*. Other wild species are also known to carry *Ry* genes including *S. stoloniferum* (Cockerham 1943), *S. brevidens* (Pehu et al. 1990), and *S. chacoense* (Hosaka et al. 2001). Recently, a hypersensitive response gene, *Ny*, conferring resistance was also identified and mapped (Szajko et al. 2014).

Marker-Assisted Resistance Breeding

In potato, molecular markers have been developed and successfully tested for a gene conferring extreme resistance to PVY. Kasai et al. (2000) developed a sequence characterized amplified region (SCAR) marker to the PVY resistance gene Ry_{adg}. The marker was generated only in genotypes carrying Ry_{adg}, when tested on 103 breeding lines and cultivars with diverse genetic backgrounds (Kasai et al. 2000). Other known R-genes that are tagged with molecular markers can be conveniently used in MAS as well. For example, breeding program markers linked to the Ns gene conferring resistance to PVS are currently being used for indirect selection in diploid. Recently, Gebhardt et al. (2006) elegantly demonstrated how MAS could be efficiently used in resistance breeding programs. The authors applied screening with PCR-based molecular markers to develop breeding material that carries a combination of four resistance genes: Ry_{adg} for extreme resistance to PVY, *Gro*1 for resistance to *G. rostochiensis*, *Rx*1 for extreme resistance to PVX, or Sen1 for resistance to potato wart. When tested in the presence of a pathogen, all selected plants showed an expected resistant phenotype. However, an important requirement for molecular markers used in MAS is their universality in a wide gene pool, not just in a specific cross. After detecting and tagging enough resistance loci, the MAS will facilitate the more efficient development of new potato cultivars carrying a desirable resistance gene combination (see review by Tiwari et al. 2012).

1.2.7 Pest Resistance

A wide range of pest resistance has been identified in wild species. Various studies indicate that resistance to insects are due to glycoalkaloids, glandular trichomes,

and other undetermined mechanisms (Pelletier et al. 2013; Flanders et al. 1992). Flanders et al. (1992) evaluated 100 species of wild potato for resistance to various insects and reported that resistance was associated with glycoalkaloid tomatine, dense hairs, and glandular trichomes. Jansky et al. (2009) reported resistance to Colorado potato beetle was confirmed in species characterized by high levels of glycoalkaloids (*S. chacoense*) or dense glandular trichomes (*S. polyadenium* and *S. tarijense*). *S. hougasii* showed high levels of resistance to Columbia root-knot nematode. Cyst nematode resistance has been identified in the Argentinian wild species *S. vernei* and *S. acaule* (Hawkes 1994).

1.2.7.1 Mapping Plant Resistance Genes

The mapping of plant resistance genes is typically carried out on segregating populations derived from parents with contrasting phenotypes. To localize genes associated with particular resistance on a molecular linkage map, the resistance phenotype has to be assessed for the individuals in the mapping population. Then, the linkage between marker loci and the resistance trait is calculated. Unfortunately, the cultivated potato (*S. tuberosum* ssp. *tuberosum*) is a highly heterozygous autotetraploid $2n = 4x = 48$ species with a complex genetic inheritance that complicates gene mapping. To limit the complexity of potato genetics, diploid $2n = 2x = 24$ individuals are frequently used as parents for molecular map construction and linkage analysis. Diploids can be derived from tetraploid genotypes through anther or pollen culture, or through interspecific hybridization with certain genotypes of *Solanum phureja* ($2n = 2x = 24$). The large population size allows the detection of flanking markers that are more closely linked to the resistance gene. Closely linked markers then may be used for either MAS or the map-based cloning of the resistance gene

Two common types of single-gene resistance to viruses in potato are hypersensitive resistance and extreme resistance. The genes for hypersensitive resistance are often virus strain specific. When plants carrying these genes are inoculated with viruses, they usually develop either local necrotic lesions in the infected tissue or systemic necrosis. Several genes coding hypersensitive resistance to potato viruses A, S, X, and Y have been mapped in potato. On the contrary, very limited (or no) necrosis is observed on plants having genes for extreme resistance. The extreme resistance genes confer comprehensive resistance to several virus strains, and only an extremely low level of virus can be detected in some of the inoculated plants. Genes for extreme resistance to PVX and PVY originating from at least four different potato species have been placed on the potato molecular map. Quantitative resistant loci (QRL) for resistance to PLRV have also been reported in some mapping progenies. Resistance genes to three economically important species of nematodes have been mapped in potato. Two of the species (*Globodera rostochiensis* and *G. pallida*) are root cyst nematodes, whereas *M. chitwoodi* is a root-knot nematode. After the discovery of the first nematode resistance gene (H1) in the 1950s, it has been introgressed into many commercially available cultivars to control *G. rostochiensis* pathotypes. The

gene is located on potato chromosome 5. Additional dominant genes for qualitative resistance to *G. rostochiens*is and *G. pallida* have been mapped, together with several major QRL. However, only a single resistance locus against *M. chitwoodi* species has been identified so far in the potato genome. The RMc1 gene from *S. bulbocastanum* was introgressed into the cultivated potato by somatic hybridization. Quantitative resistance loci against bacteria *E. carotovora* ssp. *atroseptica*, a causal agent (together with other *Erwinia* species) of potato blackleg and tuber soft rot, were detected in a diploid population with a complex pedigree that included three Solanum species: *Solanum yungasense*, *S. tuberosum*, and *S. chacoense*. Genetic factors affecting resistance to *E. carotovora* ssp. *atroseptica* were found on all 12 potato chromosomes (Zimnoch-Guzowska et al. 2000).

Very little information has been published concerning natural insect resistance loci in potato. In one study, two reciprocal backcross *S. tuberosum* × *S. berthaultii* potato progenies were screened for resistance to Colorado potato beetle (CPB) consumption, oviposition, and defoliation. Most of the quantitative resistance loci (QRL) for resistance to CPB were linked to the loci for glandular trichome traits. However, a relatively strong and consistent QRL for trichome-independent insect resistance was observed in both backcross populations on chromosome 1. In addition to resistance against *Phytophthora*, the Solanaceae family shows a conserved position of genes conferring resistance to some other pathogens. Three potato genes encoding resistance to PVY (Ry_{adg} and Ry_{sto}) and PVA Na_{adg} reside in the resistance gene hotspot on the long arm of chromosome 11 (Brigneti et al. 1997).

1.2.8 Nutrient Use Efficiency

Nutrient efficient plant plays a major role in increasing crop yields in the face of increasing climate change and global warming. At least 60% of the world's arable lands have a mineral deficiency or elemental toxicity problems. Nutrient use efficiency (NUE) defines as "the plant growth, physiological activity, yield or harvested yield per unit of nutrient". The productivity of the plants depends essentially on the nutrient balance and biological activity.

1.2.8.1 Physiological Components of NUE

In most crops, young developing leaves and roots behave as sinks for inorganic N uptake during the vegetative stage for synthesis and storage of amino acids via the nitrate assimilation pathway. These amino acids are further utilized in the synthesis of proteins and enzymes involved in different biochemical pathways and the photosynthetic machinery governing plant growth, architecture, and development. During the reproductive stage, the increased supply of nitrogenous compounds is necessary for optimum flowering and grain filling. At this stage, both N assimilation and remobilization become critical and the leaves and shoots act as the source providing amino

acids to the reproductive and storage organs (Kant et al. 2011). During tuber bulking in potato, there is also intensive reallocation of dry matter to tubers. Vos (1999) mentioned that the balance between the relative sink strengths for the nitrogen of canopy and tubers defines the carbon that can be produced in the plant because it is strongly related to the senescence process: the higher the nitrogen reallocation to tubers, the faster the canopy senescence. Mustonen et al. (2008) mentioned that tuber yield and tuber nitrogen accumulation at plant maturity were related to crop nitrogen supply and that most of the nitrogen is reallocated to tubers; it would imply that tuber nitrogen uptake is representative of the total plant nitrogen uptake. NUE has been studied in potato, in general using small numbers of genotypes or varieties. A study reported that an increase in N input induced a decrease in the agronomic NUE (i.e., amount of tubers produced per amount of nitrogen supplied), with no difference due to plant growth type. Significant variation in NUE characteristics among genotypes and across contrasting environments enhances the importance of screening-adapted potato germplasm with respect to N use efficiency characteristics based on precision phenotyping in aeroponics (Tiwari et al. 2019) (Fig. 1.1). Very recently, Tiwari and coworkers (2018) discussed an integrated approach to improve nitrogen use efficiency in potato applying genomics, breeding, and physiological approaches.

Modification of Root System Architecture (RSA) to Increase NUE

Recent and past advances in understanding RSA have come from the studies on the model plant (*Arabidopsis thaliana*) and the description of the cellular structure laid the foundation for developmental and genetic work in cereals and other well-studied crops (Smith and De Smet 2012). In potato crops, root secondary growth followed by starch deposition and increase in root biomass determines the harvestable agronomic yield. This particular area of research has not been extensively studied in potato crop

Fig. 1.1 Precision phenotyping of potato plants in aeroponic culture for roots and shoots

under nutrient deficiencies and merits research. For example, the formation of root cortical aerenchyma (RCA) in different crop species is one of the latest advances in our understanding of the impact of nutrient deficiencies in root architecture. RCA is defined as tissue with large intercellular spaces in the root cortex normally produced in plant species under hypoxia. However, RCA can be also formed in response to drought and edaphic stresses such as N and S deficiencies (Zhu et al. 2010). In maize, genotypes with greater RCA had greater topsoil foraging, P acquisition, growth, and yield under low P environments (Galindo-Castañeda et al. 2018). Currently, there are no published studies on the formation of RCA in potato crops. Another important change in root architecture as a result of nutrient deficiency is the presence or absence of root secondary growth. In potato, it has been determined that RSA traits such as specific root length of basal roots and total root weight for various root classes are related to the final tuber yield (Wishart et al. 2013). Basal roots are important for water uptake and anchorage, whereas stolon roots are connected with the nutrient acquisition and tuber formation (Wishart et al. 2013). An earlier work by Sattelmacher et al. (1990) provided evidence that root length and surface area was important for nitrogen acquisition and that a large root system was associated with higher N acquisition.

Despite these efforts, the link between storage root/tuber yield and the carbon partitioning to other root types as well as the regulatory networks is yet to be established (Khan et al. 2016). However, the cumulative evidence supporting the link between RSA and storage root in sweet potato and between RSA and tuber yield in potato paves the way forward for more in-depth work in sweet potato and potato. One way forward to overcome these barriers is to use the sweet potato (dicot, storage root), cassava (dicot, storage root), potato (dicot, tuber), and yam (monocot, tuber) as primary model systems for understanding the connection between RSA and agronomic yield in root and tuber crops, respectively. Finally, international agricultural research centers, as well as national institutions that have mandates in tuber crops, should continue to intensify RSA research investments into their current and future research priorities, especially under the threat of climate change, vulnerable agroecological landscapes and poverty. During the first Green Revolution, improved rice and wheat varieties were rapidly adopted in tropical and subtropical regions that had good irrigation systems or reliable rainfall (Evenson and Gollin 2003). The spread of these improved varieties was associated with the activity of international agricultural research centers (Evenson and Gollin 2003). It has been suggested that a second Green Revolution, one that incorporates RSA traits, is vital to improving the yield of crops grown in infertile soils by farmers with little or no access to fertilizers (Lynch 2007). Just like the first Green Revolution, such research centers will likely have an important role in ushering in the second Green Revolution (Zeigler and Mohanty 2010).

1.2.8.2 Nutriomics and NUE

The current breeding efforts are mainly implemented through a simple selection of biomass or yield in the field. Biomass or yield selection in the field is not only costly but also subject to confounding environmental interaction and spatial heterogeneity. Therefore, it would be preferable to identify and select specific traits that are directly related to a specific nutrient efficiency. Once clearly identified, these traits could be used for more efficient screening in controlled environments or tagged with molecular markers and improved marker-assisted selection or gene transformation (Yan et al. 2006). Useful traits for nutrient efficiency may be associated with altered physiological and biochemical pathways in adaptation to nutrient stress. Specific-nutrient signaling pathways, such as Pi signaling and their regulatory systems in plants, have been revealed making it feasible to modify some key regulators to enhance the uptake and use efficiency of the nutrient through genetic engineering. However, the systematic mechanism might be involved in adaptation to nutrient stress at the whole level (Yan et al. 2006). The fact that many of the molecular and biochemical changes in response to nutrient deficiency occur in synchrony suggests that genes involved are coordinately expressed and share a common regulatory system. Therefore, systematic studies are needed to understand the genomics, transcriptomics, proteomics, and metabolomics aspects of nutrient efficiency. This area of study is termed plant nutriomics, a new frontier of plant biology that is attracting more and more attention by researchers worldwide. Development of nutriomics in relation to nutrient-dense potato is becoming an imperative issue for human health. The role of functional genomics is essential to understand metabolic pathways and regulatory mechanisms of related genes in developing nutrient-rich potato.

1.2.9 Nutrient Contents in Potato

In terms of nutrition, potato is a complex source of nutrients (vitamins, carotenoids, antioxidant phenolics, proteins, magnesium, etc.), and some antinutrients (primarily glycoalkaloids). On average, potato tubers contain 77% water, 20% carbohydrates, and less than 3% of proteins, dietary fiber, minerals, vitamins, and other compounds (Zaheer and Akhtar 2016). Several breeding and molecular approaches have been employed for trait improvement in potato. Conventional breeding techniques for potato improvement are directed to increase yield, processing, and storage quality (Halterman et al. 2016). Although conventional breeding has been successfully employed for targeted trait improvement with less intraspecific variability, the progress is relatively slow and limited due to the phenotypic characterization of leading individuals in successive generations. High heterozygosity and tetraploid nature of the potato genome are major drawbacks in breeding efforts to improve potato because of allelic suppression at each breeding cross (Lindhout et al. 2011). In this context, new breeding technologies offer a leading hand for trait improvement in crop plants and provide a platform for precise and robust plant genome editing.

1.2.9.1 Increased Protein Content

Increased protein content was achieved through the constitutive expression of tuber-specific gene, *Amaranthus hypochondriacus*1 (*AmA1*) (Chakraborty et al. 2010). In transgenic potato, the enhanced protein (albumin) localizes inside cytoplasm/vacuole. The tubers of seven engineered potato cultivars showed an increased protein content of up to 60% as compared to controls (Chakraborty et al. 2010). By using RNAi technology, overexpression of an exogenous gene *Arabidopsis thaliana* cystathionine γ-synthase (AtCGS), along with the suppression of a host gene *S. tuberosum* methionine γ-lyase (StMGL), resulted in nearly a double concentration of free methionine inside transgenic tubers as compared to control tubers (Kumar and Jander 2017).

1.2.9.2 Increased Vitamin and Carotenoid Contents

RNAi approach was utilized to silence the β-carotene hydroxylase (*bch*) gene that showed a significant increase in β-carotene and lutein contents in the tubers (Van Eck et al. 2007). Another study reported a 20-fold increase in tuber carotenoid contents by expressing three bacterial genes involved in carotenoid biosynthesis (Diretto et al. 2007). Similarly, transgenic potato cv. Taedong Valley was produced, overexpressing GLOase gene (L-gulono-γ-lactone oxidase from rat cells) that showed an enhanced (141%) content of L-Ascorbic acid (vitamin C) (Upadhyaya et al. 2010).

1.2.9.3 Increased Phenolic Contents

Tuber-specific constitutive expression of an exogenous gene, flavonol-specific transcriptional activator (AtMYB12: derived from A. thaliana) showed a significant increase (>3-folds) of CQAs and total flavonoid content. The increased phenolic contents being imposing health benefits also induce some antimicrobial properties to plants, particularly with reduced fungal infections (Li et al. 2016).

1.2.9.4 Reduction of Antinutrient Contents in Potato

RNAi-mediated silencing of the host gene, *Glycoalkaloid metabolism 4* (*GAME4*), in potato showed a significant decrease (up to 74-fold) in SGAs (Solanum Glycoalkaloids) content in leaves and tubers (Itkin et al. 2011). In another study, RNAi-mediated simultaneous silencing of potato asparagine synthetase genes (StAS1 and StAS2) and VInv gene significantly reduced the CIS process as well as asparagine content in transgenic potato cv. Russet Burbank (Zhu et al. 2016). The first-generation biotech potato (Simplot's Innate TM) was engineered to have lower reducing sugar levels and reduced asparagine contents to address the acrylamide forming problems during potato frying (Halterman et al. 2016). Several studies have demonstrated

the incorporation of nutritional traits in potato such as enhanced protein content (Chakraborty et al. 2010), vitamin C content, β-carotene level (Li et al. 2012), triacylglycerol (Hofvander et al. 2016), tuber methionine (Kumar and Jander 2017), and amylose content (Krunic et al. 2018). Recently, the emergence of NBTs such as TALENs, ZFNs, and CRISPR/Cas9 has provided opportunities for robust, precise, and site-specific genome editing to introduce important agronomical traits in various crop plants. The new breeding technologies (NBTs) offer fast-track development of commercial potato cultivars such as Russet Burbank, Désirée, and Kathadin with superior traits such as improved nutrition, biotic, and abiotic stress tolerance, and enhanced yield.

1.3 Genetic Resources of Climate-Smart Genes

Genetic resources offer a vast reservoir of important novel traits and allelic variation for traits. The value of germplasm is determined by its genetic diversity, availability, and utility. In this sense, potato stands out among all other crops. The high utility of wild and landrace potatoes has led to a series of collection expeditions in the centers of origin, which are currently available for breeders and researchers through gene banks. Approximately 98,000 accessions are currently conserved ex situ and 80% of them are maintained in 30 key collections. Within these, 23,834 potato accessions are registered in GENESYS (https://www.genesys-pgr.org/ accessed Feb 24, 2019). A list of major genebank holders of ex situ collections of the potato is available (Machida-Hirano 2015). These materials are conserved either as botanical seeds or in a vegetative form (tubers and in vitro plantlets).

Cultivated potato and its wild relatives belong to the genus *Solanum;* a considerable number of highly diverse species exist in the genus with 1,500–2,000 species (PBI Solanum Project 2014). Primitive forms of cultivated potato and their wild relatives provide a rich, unique, and diverse source of genetic variation, which could be a source of various traits for potato breeding. Wild potatoes have been used in breeding programs for disease resistance, particularly LB for over 100 years (Hawkes 1994). Potato has many wild relatives and primitive cultivars and these genetic resources have proven to be valuable in breeding programs in addition to disease resistance, environmental tolerance, and other agronomic traits and processing qualities of interests (D'hoop et al. 2008; Hawkes 1994). Native Andean potatoes were shown to have a wide genetic diversity (Monte et al. 2018) along with valuable phenotypic traits, such as cold sweetening resistance (Colman et al. 2017). Sources of resistance have been screened, identified, and listed by several authors (Hawkes 1994). The data resources on wild and cultivated potato species carrying useful traits were reviewed by Machida-Hirano (2015).

The potential for using these genetic resources in conventional breeding depends on their crossability with the commonly cultivated potato *S. tuberosum*. The potato has prezygotic and postzygotic hybridization barriers, such as differences in the endosperm balance number (EBN) and ploidy level. The success of the use of wild

relatives for genetic improvement relies a lot on their crossability with cultivated species. The EBN is a strong prezygotic crossing barrier which explains the success or failure of intraspecific crosses. It relates to a strong isolating mechanism present in section *Petota*. In potato, the EBN classification are 2x (1EBN), 2x (2EBN), 4x (2EBN), 4x (4EBN), and 6x (4EBN). The EBN is independent of the ploidy level and is determined based on cross compatibility using standard EBN test crosses. Excluding other crossing barriers, hybridization is frequently successful between species with matching EBNs rather with different EBNs, regardless of ploidy. People conducting research on potatoes have developed methods for overcoming this hybridization barrier, such as ploidy manipulations, bridge crosses, auxin treatments, mentor pollinations, and embryo rescue (Machida-Hirano and Niino 2017).

1.4 Gene Transfer Through Genetic Manipulation

1.4.1 Ploidy Manipulation

The generation of haploid potatoes was demonstrated through the use of *Solanum phureja* ($2n = 2x = 24$) as a pollen donor onto *S. tuberosum* and haploids are now relatively easy to generate. Due to the complex nature of genetic interactions among genes in a tetraploid species, haploid potatoes are desirable for the study of genetic interactions in a less complex background. A breeding scheme involving the scaling up and down of potato chromosome sets was referred to as analytical breeding. The first step involves the generation of maternal haploids from tetraploid cultivars through parthenogenesis by crossing to a diploid species. The resulting plants, often referred to as dihaploids in potato, are crossed with other diploid stocks for breeding at the diploid level. The final step involves returning to the tetraploid level by unilateral (or bilateral) sexual polyploidization using 4x 2x (or 2x2x) crosses and relying on the occurrence of unreduced 2n gametes in the diploid parent. Such cycling between ploidy levels is achieved relatively easily in potato and provides a means to simplify the introgression of traits from new sources of genetic diversity. The use of a haploid genotype was pivotal in the potato genome sequencing project (Potato Genome Sequencing Consortium 2011).

1.4.2 Embryo Culture

The endosperm balance number (EBN) plays an important role in the speciation of tetraploid from diploid Solanum species. Upon hybridization, the EBN should be in a 2:1 maternal to the paternal ratio for normal endosperm development and successful seed production. The hybridization barriers between disomic (2 EBN) and tetrasomic

(4 EBN) tuberous Solanum species can be overcome by double pollination and rescue of aborting embryos via tissue culture. Embryo culture has also been valuable for circumvention of other forms of interspecific incompatibility. For example, resistance to potato leafroll virus was successfully introgressed from *S. etuberosum* to *S. tuberosum* via embryo culture. An extreme example of the use of embryo culture to aid the recovery of wide hybrids involves the successful hybridization of the disomic hexaploid *S. nigrum* (black nightshade) as a female parent with tetraploid potato.

1.4.3 Somaclonal Variation

During the history of regenerating plants from cell cultures, the perceptions of the clonal integrity of the resulting plants have changed. The historical dogma was that all plants regenerated from somatic tissue are identical to the parent plant. However, the contrary view was popularized by Larkin and Scowcroft (1981), who described the high frequency of phenotypic variants observed among those regenerated plants from cell culture and coined the term "somaclonal variation" for the phenomenon. For the "optimists", somaclonal variation was seen as a new approach for generating novel variation in plants, especially in clonally propagated crops such as potato. For the "pessimists", it was seen as an inherent curse for other applications of cell culture for crop improvement.

The observation that phenotypic changes arise during the cell culture and regeneration phase of potato tissue culture was communicated more widely following the recovery of a vast array of novel phenotypes after regeneration of plants from leaf protoplasts of "Russet Burbank" and similar cultivars. This finding was quickly given support from other studies that involved growing potato somaclones in the field. Explanations that account for the observed phenotypic changes among somaclonal potato lines involve physiological, epigenetic, or genetic changes associated with the cell culture and shoot regeneration phase of plant transformation. As not all variants have a genetic basis, lines exhibiting phenotypic changes need to be grown over several field seasons to ensure the stability of performance. Stable phenotypic changes of either heritable and/or epigenetic origin may arise through ploidy changes, chromosomal aberrations, gene amplification, activation of transposable elements, DNA methylation changes, or point mutations and can also occur during the long-term propagation of potato from internodal stem cuttings. Phenotypic variation among plants regenerated from cell cultures is often correlated with changes in chromosome number and/or structural chromosome aberrations, and such changes have been frequently observed in regenerated potato plants. Such cytological changes are usually accompanied by a poor agronomic performance.

At the time, it was widely believed that these variants arising in cell culture could be applied immediately as single trait improvements to existing elite cultivars already well established in the market place. Considerable effort was devoted to recovering useful variants in a wide range of cultivars, and variants with improvements in specific

useful traits were reported. However, it has been recognized over time that the recovery of a somaclonal line exhibiting beneficial traits without others simultaneously arising negative attributes is very rare. Nowadays, the phenomenon of somaclonal variation is widely seen as an inherent negative feature of regeneration from cell culture and considered as something to be avoided. Strategies to minimize the impact of somaclonal variation on plant performance are routinely implemented during other applications of cell culture for potato improvement.

1.4.4 Somatic Hybridization and Protoplast Culture

By the early 1980s, the routine isolation and culture of protoplasts were possible for many plants using cell wall degrading enzymes, coupled with maintaining the appropriate osmotic balance until cell walls had redeveloped. Upon the mixing of protoplasts from different species, somatic fusion can be stimulated by chemical or electrical treatments prior to the re-synthesis of cell walls. The regeneration of somatic hybrid plants from these cells is possible provided the two parental species are closely related, even if they cannot be sexually hybridized. Such somatic hybrid plants offer new sources of germplasm for the introgression of traits into crop plants, although this is often very challenging due to the poor fertility of the initial somatic hybrids (review by Tiwari et al. 2018).

Somatic hybridization has provided some new opportunities for introgression of novel sources of disease and pest resistance into cultivated potato from accessions of taxa possessing sexual reproductive barriers with potato. Resistances to diseases caused by leaf roll virus, potato virus Y, early and late blight, soft rot, Columbia root-knot nematode, and Colorado potato beetle have been introduced through somatic fusion of potato protoplasts with protoplasts of wild relatives, including *S. pinnatisectum* (Sarkar et al. 2011), *S. etuberosum* (Tiwari et al. 2010), *S. cardiophyllum* (Chandel et al. 2015) to name a few (review by Tiwari et al. 2018). Somatic hybrids have been utilized to identify new genes by microarray (Tiwari et al. 2015) and organelle genome analysis (Tiwari et al. 2014). However, despite these hybrids being good sources of resistance to pathogens, as well as abiotic stress, they often produce small misshapen tubers that are far from suitable for agricultural production. Multiple cycles of backcrosses are required for these plants to be useful in agriculture.

The regeneration of plants from protoplast cultures offers nowadays another advantage linked to the delivery of the genome-editing machinery (ribonucleases) avoiding DNA integration in the host genome, as it will be presented below.

1.5 Gene Transfer Through Genetic Engineering

1.5.1 Transgenic

Potato was one of the first crops for which transgenic plants were developed. The use of genetic engineering approaches has allowed the successful transfer of numerous transgenes into elite potato cultivars including pest and disease resistances; abiotic stress resistance; quality attributes for improved processing, nutrition and appearance; and novel products for biopharming. This is virtually impossible via traditional breeding due to the high heterozygosity in the tetraploid potato genome. As a consequence, potato transformation represents the only effective way to produce isogenic lines of specific genotypes/cultivars. Although this is not an exhaustive list, it clearly highlights the diversity of traits successfully transferred to potato by genetic transformation and illustrates the immense potential of transformation for the genetic improvement in potato.

GM potato "NewLeaf" expressing the Cry3A toxin was released by Monsanto against Colorado potato beetle (CPB). They were commercially available in the USA from 1996 to 2000 and provided good CPB control, but were later discontinued following perceived concerns from consumers, marketing issues, and the introduction of a novel insecticide that controls both beetles and aphids. Against Lepidoptera, such as PTM, the Cry1 class of the Bt genes has been shown to be highly effective, for example, cry1Ab2 (Chakrabarti et al. 2000). Chimeric Bt cry genes comprising domains I and II from cry1Ba and domain II of cry1Ia were developed to act on a wider spectrum of insect species. Transgenic potato plants were resistant to insect pests from two different orders—Coleoptera (CPB) and Lepidoptera (PTM and European corn borer) (Naimov et al. 2003). Transgene pyramiding of the cry1Ac9 and cry9Aa2 genes has been achieved in clonal crop potato, where sexual hybridization to pyramid transgenes is unsuitable. Transformation of potatoes with a range of Bt-based transgenes has proven to be a highly successful approach to controlling insect pests of potato.

1.5.2 Intragenics and Cisgenics

Despite the rapid global adoption of GM technology in agricultural crops including potato, many concerns have been raised about transgenic crops (James 2010). One of the main underlying sources of concern involves the transfer of genes across very wide taxonomic boundaries, for example, the insertion of bacterial genes into plant genomes (Lammerts van Bueren et al. 2007). The widespread application of transgenic techniques in comestible plants raised public concerns mainly about health safety although there is no scientific evidence that genetically modified crops harm human health (Kamthan et al. 2016). Nevertheless, its use continues to be a topic of debate due to questions concerning intellectual property and biosafety issues

involved in open field planting. To surmount these deficiencies, another generation of GM technology is being developed, known as cisgenic and/or intragenic crops. In contrast, this methodology allows the transfer of only natural genes from the same or crossable species (Ricroch and Hénard-Damave 2015).

Any gene from a wild relative that confers a trait of interest has the potential to improve elite potato germplasm through cisgenics/intragenics. For example, in potato (Solanum tuberosum), the asparagine synthase-1 (StAst1) gene was silenced following the intragenesis concept with the aim of reducing the formation of acrylamide in potatoes during baking and frying (Chawla et al. 2012). The silencing vector comprised gene elements of different potato genes. After the selection of intragenic potato lines, these were field tested and the resulting tubers showed a 70% reduction in acrylamide levels after processing. In 2014, the US Department of Agriculture (USDA) approved the deregulation of an intragenic potato line in which, in addition to StAst1, the polyphenol oxidase-5 gene was also silenced for the prevention of enzymatic browning caused by bruising and exposure to oxygen after peeling or cutting, thus allowing the cultivation of this potato in the USA. This potato strain was also tested by the US Food and Drug Administration (FDA) for food and feed safety.

Cisgenesis may become an important approach to introduce broad-spectrum potato late blight resistance into elite susceptible crop cultivars, especially when the focus is on stacking multiple resistance genes. Resistance genes to late blight and scab originating from crop wild relatives have been used to produce cisgenic late blight-resistant potato and scab-resistant apple varieties, respectively. The performance of several cisgenic potato lines with late blight resistance genes originating from different wild species has been known Cisgenesis approach to introduce two, *Rpi-sto1* and *Rpi-vnt1.1* genes from the crossable species *Solanum stoloniferum* and *Solanum venturii*, respectively, into three different potato varieties was reported (Jo et al. 2014).

1.5.3 Genome Editing

Genome editing is a method that enables specific nucleotides in the genome of an individual to be changed. Genome-editing technologies such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and—more recently—CRISPR system, provide an unprecedented advancement in genome engineering due to precise DNA modification. This latter method has proved experimentally accessible in a broad range of organisms. Its use is based on the introduction of an endonuclease (usually from *Streptococcus pyogenes*), guided by an RNA sequence to induce a cut in specific regions of the DNA. The molecular process of repairing is then exploited to promote desired variations in the DNA sequence (Ding et al. 2016). For heterozygous, polyploid and vegetatively propagated crops such as cultivated potato, *Solanum tuberosum* Group Tuberosum L., genome-editing presents

tremendous opportunities for trait improvement with the potential produce incremental improvements in already established elite cultivars, similar to autogamous crop breeding (Feingold et al. 2018).

In potato, traits such as improved resistance to cold-induced sweetening, processing efficiency, herbicide tolerance, modified starch quality, and self-incompatibility have been targeted utilizing CRISPR/Cas9 and TALEN reagents in diploid and tetraploid clones. For example, Vacuolar invertase (*StVlnv*) gene that associates with cold-induced sweetening, increasing acrylamide content in tubers has been targeted through genome editing using TALENs for improved cold storage (Clasen et al. 2016). CRISPR/Cas9 was used Transient expression of CRISPR/Cas9 targeting Protoplasts *Granule-bound starch synthase (StGBSS)* in potato protoplasts for targeted mutagenesis and regeneration, tuber with altered starch content was developed. Knockout of self-incompatibility gene *S-RNase* in diploid potato line resulted in self-compatibility through CRISPR/Cas9 (Ye et al. 2018). However, their application on plant biotechnology is still facing the same challenges to insert the molecular components for genome editing is being widely applied in plants and has revolutionized crop improvement. In this regard, there are other ways for plant genome editing such as through zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and more recently with CRISPR-Cas9 system. This implies that new varieties could be developed much faster than usual traditional or even molecular breeding methods. In addition, GE technology is also very useful for generating targeted variations, thereby broadening the allele pool for precision breeding (Scheben et al. 2017). The enormous potential of this technique relies on the identification of key responsible genes (and their naturally occurring variants) for improving traits. Most importantly, the resultant product of genome editing, as per the scientific community, is not a genetically modified organism (GMO) (Huang et al. 2016), a regulatory position that is accompanied by countries of North, Central, and South America (Feingold et al. 2018). Therefore, the GE approach, along with its superior and much more precise features over transgenesis, is under the same regulatory requirements as cultivars obtained by conventional breeding if no foreign DNA is integrated into the host genome. Because of this fact and since breeding objectives are focused on both producer and consumer benefits, it is likely not to face an adverse public perception.

1.6 Genetics and Genomics

Molecular technologies have huge potential for speeding up the process of conventional plant breeding. Identification of naturally existing allelic variation at the molecular level can provide a powerful tool to accelerate the process of breeding for improved cultivars. Molecular markers can be used as proxies for phenotypic characteristics of interest allowing selection of genetically elite plants in early generations. The ability to identify elite plants and discard non-elite plants saves both time and money in the process of plant breeding.

1.6.1 Molecular Genetics

1.6.1.1 Linkage Mapping

Traditionally, linkage mapping has been the most commonly used way to correlate natural variation in phenotype with genotype (Myles et al. 2009). Genetic mapping in cultivated potato has been hindered by its complex genetics. Most cultivars and breeding lines are autotetraploid and carry a high genetic load (Bonierbale et al. 1988). Tools such as (homozygous) mutant lines, recombinant inbred lines, and near-isogenic lines are not available in potato. Diploid lines derived from tetraploid *S. tuberosum*, and diploid (wild) species of potato have been used over recent decades to unravel the genetics of traits. In potato, this has been performed predominantly in segregating diploid F_1 mapping populations, established using biparental crosses of heterozygous lines. Early examples of diploid linkage maps include Bonierbale et al. (1988) who took advantage of the high level of synteny between potato and tomato and used tomato restriction fragment length polymorphism (RFLP) markers for map construction; Gebhardt et al. (1989) who used potato RFLP markers; and Jacobs et al. (1995) who combined molecular (RFLP) markers with morphological traits in one genetic map.

RFLP-based genetic linkage maps were soon followed by potato maps containing amplified fragment length polymorphism (AFLP) markers (Vos et al. 1995). Randomly amplified polymorphic DNA (RAPD) markers combined with bulked segregant analysis (BSA) have been used to identify DNA sequences linked to major gene traits of interest in potatoes (e.g., Jacobs et al. 1996). However, RAPD markers have low reproducibility and have been superseded by more reliable markers such as SSR (Simple Sequence Repeats, or microsatellites). These markers have been used since for locating SSR markers on linkage groups using segregating mapping populations, as well as for identifying and fingerprinting potato germplasm accessions and cultivars (Feingold et al. 2005; Tiwari et al. 2018). Studies using Diversity Array Technology (DArT) (Jaccoud et al. 2001) markers in potato have been published only recently; Sliwka et al. (2012) used DArT markers to map *Rpi-mch,1* a late blight resistance gene.

1.6.1.2 High-Resolution Melting Analysis (HRM)

HRM is a technology that discriminates amplicons of alleles with different haplotypes (one or multiple single nucleotide polymorphisms; SNPs) and/or can be used to detect mutations (Wittwer et al. 2003). It makes use of the small differences in melting temperatures of amplicons of double-stranded DNA molecules with one or multiple SNPs. It can be performed as a simple closed-tube assay, on DNA amplicons post-PCR without the need for separation or processing of the samples. HRM mapping relies on prior knowledge of allele sequences and haplotype variation. Although

developing HRM assays to distinguish, all alleles in tetraploid potato can be time-consuming, it has been demonstrated as an efficient genotyping system. De Koeyer et al. (2010) developed HRM assays for five molecular markers/candidate genes for genotyping and variant scanning in diploid, as well as tetraploid potato, and demonstrated that HRM-based candidate gene analysis efficiently provides information on allele dosage and discriminates different haplotypes. HRM technology was also successfully applied to examine the effect of allele dosage of the zeaxanthin epoxidase gene (*Zep*1, a recessive gene) on total carotenoid content in yellow-fleshed tetraploid potato germplasm (McCord et al. 2012).

1.6.1.3 Candidate Genes

A more direct approach to understanding the genetic inheritance of a trait of interest is the use of candidate genes. Rather than using anonymous molecular markers, genes thought to play a role in the trait of interest are investigated as candidate markers for the trait. This requires prior knowledge of the biochemical or physiological processes and pathways involved in determining a phenotype, as well as the gene sequences underpinning these processes and pathways, and can result in "perfect markers" where no recombination occurs between the marker and the trait. Chen et al. (2001) created a molecular function map for carbohydrate metabolism and transport comprising genes involved in carbohydrate metabolism.

1.6.1.4 Quantitative Trait Loci (QTLs)

Quantitative traits refer to traits that are often attributed to more than one gene controlling or influencing the observed phenotype. QTLs are regions of the genome that contain genes influencing the phenotypic expression of the trait. In potato, progeny lines from a biparental cross segregating for the trait of interest are assessed, and markers of choice are used to genotype individuals in the population. Numerous examples exist for QTL mapping in potato in both diploid and tetraploid populations. Early QTL mapping studies include using RFLP and RAPD markers to determine QTL for chip color and tuber dormancy (Freyre et al. 1994), and RFLP markers to identify QTLs for resistance to *Phytophthora infestans* (Leonards-Schippers et al. 1994). More recently, QTL analysis in combination with a candidate gene approach was successfully used by Werij et al. (2012) to analyze the genetic basis of various tuber quality traits in a diploid mapping population.

1.6.1.5 Association Mapping

Association mapping, also known as linkage disequilibrium mapping, identifies loci involved in the inheritance of complex traits by determining whether a statistically significant association exists between the genotype at a locus and the phenotype

(Mackay and Powell 2007). Association genetics is not limited to biparental crosses and can be applied in collections of breeding lines and cultivars. This is an advantage of association mapping over (QTL) linkage mapping, where the generation of segregating populations with large numbers of progeny for analysis is required. In addition, a larger pool of alleles across a genetically diverse range of lines can be assessed. Examples in potato include associations between tuber quality traits and AFLP markers (D'Hoop et al. 2008), associations between candidate gene alleles and cold-induced sweetening of potato tubers (Baldwin et al. 2011) and the association of various combinations of candidate gene alleles and their positive and/or negative effects on tuber quality (Li et al. 2013). In recent years, as larger numbers of molecular markers have become available (many based on the detection of SNPs), association mapping across the entire genome has become feasible (genome-wide association studies, GWAS; reviewed by Morrell et al. (2012).

1.6.2 Marker-Assisted Selection (MAS)

The relative lack of implementation of molecular markers in tetraploid potato breeding programs compared with some other crops is mainly due to the high level of natural allelic variation in potato, caused by the autotetraploid nature of cultivated potato and its tetrasomic inheritance (Luo et al. 2001). This high level of allelic variation hampers the ability to transfer markers across mapping populations to breeding lines, and hence, marker validation in breeding germplasm is critically important (Milczarek et al. 2011). Because mapping in tetraploid potato is far more complicated than in diploid potato, it has frequently been restricted to regions of the genome containing a trait of interest (Bradshaw et al. (2008). In addition, computer programs to assist in the genetic mapping of traits in an autotetraploid species need to eliminate many markers and/or marker alleles from analyses as the complete allelic composition and dosage remain elusive (Hackett and Luo 2003). Marker-assisted breeding has been applied in tetraploid potato for resistance to potato cyst nematode *Globodera pallida* (Moloney et al. 2010). Gebhardt et al. (2006) developed potato clones with multiple pathogen resistance traits by applying PCR-based markers to combine Ry_{adg} (resistance to PVY), *Gro*1 (resistance to the nematode *Globodera rostochiensis*) and *Rx*1 (resistance to potato virus X), or *Sen*1 (resistance to potato wart, *Synchytrium endobioticum*).

1.6.3 Genomics

The availability of potato genome has opened the possibilities of wider genomic applications for potato improvement (Potato Genome Sequencing Consortium 2011). The elucidation of the reference potato genome, including the annotation of more than 39,000 protein-coding genes, has opened up opportunities to rapidly identify

candidate genes in regions associated with a trait of interest. For example, the identification of both the StSP6A gene for tuber initiation (Navarro et al. 2011) and the StCDF1 gene responsible for plant maturity phenotype (Kloosterman et al. 2013) was greatly aided by the genome sequence. The genome sequence also provides a catalog of candidate resistance genes in the potato genome, radically enhancing our ability for rapid discovery and introgression of R-genes in potato (Lozano et al. 2012). The targeted re-sequencing of the many wild species of potato that harbor resistance to the major pests and pathogens of potato should enable the identification of a wide array of valuable resistances for breeders. A reference genetic map using a mapping population derived from the doubled monoploid "DM" was developed to assist with the anchoring of the genome sequence scaffolds to chromosomal positions (Potato Genome Sequencing Consortium 2011). This genetic map contains SNP markers as well as SSR and DArT markers. Genome sequences were anchored to the 12 linkage groups using a combination of in silico and genetic mapping data.

The single nucleotide polymorphism (SNP) frequency is very high in the potato genome (Potato Genome Sequencing Consortium 2011). An SNP chip based on the "DM" genome sequence has been developed and contains 8,303 SNPs, including many targeted to candidate genes (Hamilton et al. 2011). The positions of these SNPs on the "DM" genome are known, which allows for the rapid identification of genomic regions of interest. The first genetic maps based on diploid biparental populations using the SNP chip include over 4,400 markers and refined the anchoring data of the potato genome sequence (Felcher et al. 2012). The high frequency of SNPs in the potato genome implies that selection for favorable alleles based on one single SNP is unreliable because it may not in all cases be indicative of the desired phenotype. Effective selection is more likely to be based on haplotype selection, targeting a combination of several SNPs in one gene. This requires knowledge of the various alleles and involves (re)sequencing of all possible alleles and/or genome re-sequencing of lines to ensure all allelic/haplotype variation is represented. A major hurdle in the analysis of SNP chip data in tetraploid potato is the difficulty of scoring heterozygous allele dosage (Voorrips et al. 2011). Software such as GenomeStudio (Illumina) for analysis of SNP data was originally developed for diploid species and is currently unable to differentiate the simplex (AAAB, ABBB) and duplex (AABB) heterozygous genotypes. The development of experimental and computational methods for haplotype estimation in polyploid species is an important goal. In addition to natural allelic variation, presence/absence variation (PAV, visible as "null alleles") was found to be very common in potato, and this will present additional challenges for the application of marker-assisted selection. In addition, gene copy number variation has been shown recently to vary markedly between different potato cultivars, further highlighting the complex nature of potato genetics. As well as being powerful tools for gene discovery in their own right, genome-wide assays will provide immediate benefit to plant breeders by enabling the development of robustly unique marker haplotypes spanning QTL regions, which will be useful in both introgression breeding and whole-genome approaches such as genomic selection (Morrell et al. 2012). The availability of a genome-wide marker set polymorphic in elite germplasm will make

it possible to genotype increasing numbers of cultivars and breeding clones and will be a valuable tool for advancing whole-genome selection in potato breeding.

In addition to the potato genome sequence, RNA sequence data from 32 "DM" and 16 "RH" libraries representing all major tissue types, developmental stages and responses to abiotic and biotic stresses were generated. These provide a valuable resource that determines the expression profiles of genes of interest in different tissues, stages of growth, and in response to different growing conditions. For example, Massa et al. (2011) identified both tissue-specific gene expression profiles (including tuber-specific expression) and genes with condition-restricted expression.

With the reduction in the cost of sequencing and the concomitant increase in data output per experiment, genotyping-by-sequencing (GBS) (Elshire et al. 2011) is becoming feasible for species with a high level of diversity. The reduced representation can be achieved by sequencing libraries digested with methylation-sensitive restriction enzymes so that gene-rich regions are targeted. Alternatively, targeted resequencing of preselected genome regions can be achieved through sequence capture approaches as recently described by (Uitdewilligen et al. 2013).

The utility of the potato genome sequence for genomics-assisted breeding strategies will probably be realized in two distinct phases. Firstly, the sequence will be a powerful tool for gene discovery and placing any gene sequence into its genetic and genomic context. In the longer term, the identification of genes responsible for key agronomic traits coupled with the description of their allelic variation and their effect on the phenotype of the plant will afford breeders precision in grouping complementary alleles that will maximize the effect on the phenotype of the resulting breeding line and ultimately the cultivar.

The ability to mine an entire genome sequence is the ultimate tool for molecular breeding strategies. Many of the traits of interest to plant breeders are quantitative in nature. Even with the availability of the genome sequence, SNP chips, and GBS, the lead time for the comprehensive genetic dissection of these traits may be several years. The overwhelming amount of sequence data available to us in the near future will overshadow the amount of accurate and reliable phenotypic data necessary to advance the potato breeding efforts for traits of interest. Phenotyping has already become the limiting factor in the exploration of the genetic potential of potato.

1.7 Conclusions

To feed 9 billion people in 2050, global food needs to increase by 70%. For example, current potato production in India under optimized agricultural practices is on average 22 tons per hectare average. However, the estimated demand for various uses of potato would require an average increase of 34.51 tonnes per hectare average during 2050 under the climate change scenario. Through applications of biotechnologies such as tissue and cell culture, genetic engineering, marker-assisted technologies, genome-assisted technologies, or a combination of technologies for the improvement in potato, potato has the potential to provide an increased proportion of the food intake

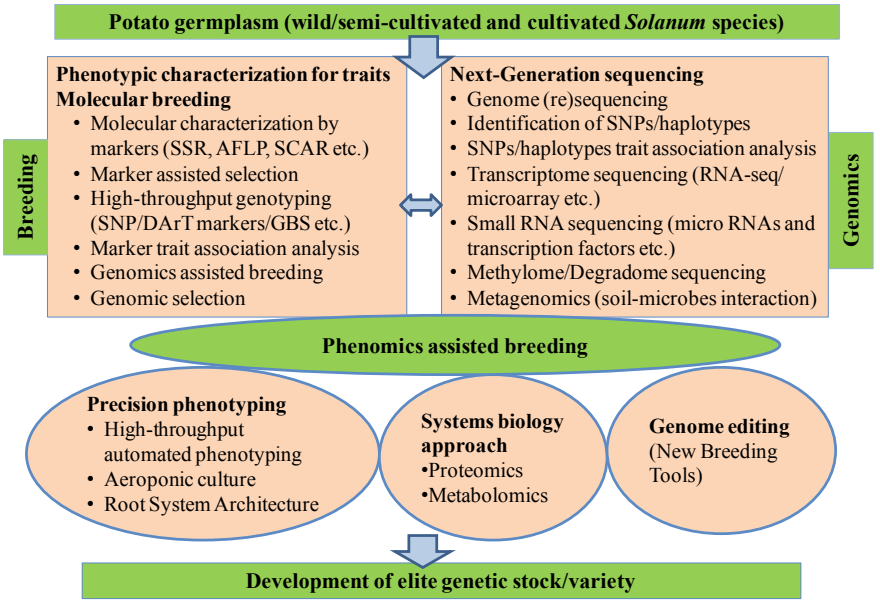

Fig. 1.2 A schematic layout showing integrated breeding-, genomics-, and phenomics-assisted approach for potato improvement

required for the anticipated population expansion over the coming decades. Access to these biotechnologies is of vital importance for developing countries. Molecular plant breeding is considered one of the most potent technologies to improve the crop yield and its productivity under climate change scenario. A layout showing an integrated breeding, genomics, and phenomics approach for potato improvement is depicted in Fig. 1.2.

Acknowledgements The Authors thank the Competent Authority, Indian Council of Agricultural Research-Central Potato Research Institute, Shimla for necessary support under the Biotechnology Program and CABin Scheme (ICAR-IASRI, New Delhi).

References

Abdullah Z, Ahmad R (1982) Salt tolerance of Solanum tuberosum L growing on saline soils amended with gypsum. Z Fuer Acker-Und PflanzBau 151:409–416

Abelenda JA, Navarro C, Prat S (2014) Flowering and tuberization: a tale of two nightshades. Trends Plant Sci 19:115–122

Anonymous (2015) CPRI Vision 2015. ICAR-CPRI, Shimla, Himachal Pradesh, India, p 50

Apse MP, Blumwald E (2002) Engineering salt tolerance in plants. Curr Opin Biotechnol 13:146–150

Baldwin SJ, Dodds KG, Auvray B, Genet RA, Macknight RC, Jacobs JME (2011) Association mapping of cold-induced sweetening in potato using historical phenotypic data. Ann Appl Biol 158:248–256

Bonierbale MW, Plaisted RL, Tanksley SD (1988) Construction of a genetic map of potato based on molecular markers from tomato. Amer Potato J 65:471–472

Bradshaw JE, Hackett CA, Pande B, Waugh R, Bryan GJ (2008) QTL mapping of yield, agronomic and quality traits in tetraploid potato (*Solanum tuberosum* subsp *tuberosum*). Theor Appl Genet 116:193–211

Brigneti G, Garcia-Mas J, Baulcombe DC (1997) Molecular mapping of the potato virus Y resistance gene *Rysto* in potato. Theor Appl Genet 94:198–203

Bukasov SM (1933) The potatoes of South America and their breeding possibilities. (According to data gathered by expeditions of the institute of plant industry to central and South America). Suppl 58 Bull Appl Bot Leningrad (1–192)

Burgutin AB, Butenko RG, Kaurov BA, Iddagoda N (1996) In vitro selection of potato for tolerance to sodium chloride. Russ J Plant Physio 1(43):524–531

Castillo JA, Plata G (2016) The expansion of brown rot disease throughout Bolivia: possible role of climate change. Can J Microbiol 62:442–448

Celebi-Toprak F, Behnam B, Serrano G, Kasuga M, Yamaguchi-Shinozaki K, Naka H, Watanabe JA, Yamanaka S, Watanabe KN (2005) Tolerance to salt stress of the transgenic tetrasomic tetraploid potato, Solanum tuberosum cv Desiree appears to be induced by the DREBIA gene and rd29A promoter of Arabidopsis thaliana. Breed Sci 55:311–319

Chakrabarti SK, Conghua X, Tiwari JK (2017) The Potato Genome. Springer, Switzerland, p 326

Chakrabarti SK, Mandaokar AD, Shukla A, Pattanayak D, Naik PS et al (2000) Bacillus thuringiensis cry1Ab gene confers resistance to potato against *Helicoverpa armigera (Hubner)*. Potato Res 43:143–152

Chakrabarti SK, Singh BP, Thakur G, Tiwari JK, Kaushik SK et al (2014) QTL mapping underlying resistance to late blight in a diploid potato population of *Solanum spegazzinii* × *S. chacoense*. Potato Res 57:1–11

Chakraborty S, Chakraborty N, Agrawal L, Ghosh S, Narula K et al (2010) Next-generation protein-rich potato expressing the seed protein gene Am A1 is a result of proteome rebalancing in transgenic tuber. Proc Natl Acad Sci USA 107:17533–17538

Chandel P, Tiwari JK, Ali N, Devi S, Sharma S et al (2015) Interspecific potato somatic hybrids between *Solanum tuberosum* and *S. cardiophyllum*, potential sources of late blight resistance breeding. Plant Cell Tiss Organ Cult 123:579–589

Chawla R, Shakya R, Rommens CM (2012) Tuber-specific silencing of asparagine synthetase-1 reduces the acrylamide-forming potential of potatoes grown in the field without affecting tuber shape and yield. Plant Biotechnol J 10:913–924

Chen L, Guo X, Xie C, He L, Cai X et al (2013) Nuclear and cytoplasmic genome components of *Solanum tuberosum* + *S. chacoense* somatic hybrids and three SSR alleles related to bacterial wilt resistance. Theor Appl Genet 126:1861–1872

Chen X, Salamini F, Gebhardt C (2001) A potato molecular-function map for carbohydrate metabolism and transport. Theor Appl Genet 102:284–295

Chinnusamy V, Zhu JK, Sunkar R (2010) Gene regulation during cold stress acclimation in plants. Methods Mol Biol 639:39–55

Christmann A, Moes D, Himmelbach A, Yang Y, Tang Y, Grill E (2006) Integration of abscisic acid signalling into plant responses. Plant Biol 8(03):314–325

Clasen BM, Stoddard TJ, Luo S, Demorest ZL, Li J et al (2016) Improving cold storage and processing traits in potato through targeted gene knockout. Plant Biotechnol J 14:169–176

Cockerham G (1943) Potato breeding for virus resistance. Ann Appl Biol 30:105–108

Colman SL, Massa GA, Carboni MF, Feingold SE (2017) Cold sweetening diversity in Andean potato germplasm from Argentina. J Sci Food Agri 97(14):4744–4749

D'hoop BB, Paulo MJ, Mank RA, van Eck HJ, van Eeuwijk FA (2008) Association mapping of quality traits in potato (*Solanum tuberosum* L.). Euphytica 161: 47–60

Danan S, Chauvin JE, Caromel B, Moal JD, Pellé R, Lefebvre V (2009) Major-effect QTLs for stem and foliage resistance to late blight in the wild potato relatives *Solanum sparsipilum* and *S. spegazzinii* are mapped to chromosome X. Theor Appl Genet 119:705–719

De Koeyer D, Douglass K, Murphy A, Whitney S, Nolan L et al (2010) Application of high-resolution DNA melting for genotyping and variant scanning of diploid and autotetraploid potato. Mol Breed 25:67–90

Ding Y, Li H, Chen LL, Xie K (2016) Recent advances in genome editing using CRISPR/Cas9. Front Plant Sci 7:1–12

Diretto G, Al-Babili S, Tavazza R, Papacchioli V, Beyer P, Giuliano G (2007) Metabolic engineering of potato carotenoid content through tuber-specific overexpression of a bacterial mini-pathway. PLoS ONE 2:e350

Dua VK, Singh BP, Govindakrishnan PM, Kumar S, Lal SS (2013) Impact of climate change on potato productivity in Punjab–a simulation study. Curr Sci 105:787–794

Dutt S, Manjul AS, Raigond P, Singh B, Siddappa S, Bhardwaj V, Kanwar PG, Patl VU, Kardile HB (2017) Key players associated with tuberization in potato: potential candidates for genetic engineering. Crit Review Biotechnol 37:942–957

Elkhatib HA, Elkhatib EA, Allah AMK, E-Sharkawy AM (2004) Yield response of salt-stressed potato to potassium fertilization: a preliminary mathematical model. J Plant Nutrit 27:111–122

Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K et al (2011) A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS ONE 6:e19379

Evenson RE, Gollin D (2003) Assessing the impact of the green revolution 1960 to 2000. Science 300:758–762

Ewing EE, Simko I, Smart CD, Bonierbale MW, Mizubuti ESG et al (2000) Genetic mapping from field tests of qualitative and quantitative resistance to *Phytophthora infestans* in a population derived from *Solanum tuberosum* and *Solanum berthaultii*. Mol Breed 6:25–36

FAO (2013) http://www.fao.org/publications/sofa/2013/en/

Feingold S, Bonnecarrère V, Nepomuceno A, Hinrichsen P, Cardozo Tellez L, et al (2018) Edición génica: una oportunidad para la región. Rev Investigac Agropecuar 44(424):427. Felcher KJ, Coombs JJ, Massa AN, Hansey CN, Hamilton JP et al (2012) Integration of two diploid potato linkage maps with the potato genome sequence. PLoS One 7: e36347

Feingold S, Lloyd J, Norero N, Bonierbale M, Lorenzen J (2005) Mapping and characterization of new EST-derived microsatellites for potato (*Solanum tuberosum* L.). Theor Appl Genet 111:456–466

Fidalgo F, Santos A, Santos I, Salema R (2004) Effects of long-term salt stress on antioxidant defence systems, leaf water relations and chloroplast ultrastructure of potato plants. Ann Appl Biol 145:185–192

Finkelstein RR, Gampala SSL, Rock CD (2002) Abscisic acid signaling in seeds and seedlings. Plant Cell 14:S15–S45

Flanders KL, Hawkes JG, Radcliffe EB, Lauer FI (1992) Insect resistance in potatoes: sources, evolutionary relationships, morphological and chemical defenses, and ecogeographical associations. Euphytica 61:83–111

Fock I, Collonnier C, Luisetti J, Purwito A, Souvannavong V et al (2001) Use of *Solanum stenotomum* for introduction of resistance to bacterial wilt in somatic hybrids of potato. Plant Physiol Biochem 39:899–908

Fock I, Collonnier C, Purwito A, Luisetti J, Souvannavong V et al (2000) Resistance to bacterial wilt in somatic hybrids between *Solanum tuberosum* and *Solanum phureja*. Plant Sci 160:165–176

Foster SJ, Park T-H, Pel MA, Brigneti G, Śliwka J et al (2009) *Rpi-vnt*1.1, a Tm-22 homolog from *Solanum venturii* confers resistance to potato late blight. Mol Plant Microbe Interact 22:589–600

Freyre R, Warnke S, Sosinski B, Douches DS (1994) Quantitative trait locus analysis of tuber dormancy in diploid potato (*Solanum* spp). Theor Appl Genet 89:474–480

Galindo-Castañeda T, Brown KM, Lynch JP (2018) Reduced root cortical burden improves growth and grain yield under low phosphorus availability in maize. Plant, Cell Environ 41:1579–1592

Gebhardt C, Bellin D, Henselewski H, Lehmann W, Schwarzfischer J, Valkonen JP (2006) Marker-assisted combination of major genes for pathogen resistance in potato. Theor Appl Genet 112:1458–1464

Gebhardt C, Ritter E, Debener T, Schachtschabel U, Walkemeier B et al (1989) RFLP analysis and linkage mapping in *Solanum tuberosum*. Theor Appl Genet 78:65–75

Ghosh SC, Asanuma K, Kusutani A, Toyota M (2001) Effect of salt stress on some chemical components and yield of potato. Soil Sci Plant Nutr 47:467–475

Hackett CA, Luo ZW (2003) TetraploidMap: construction of a linkage map in autotetraploid species. J Hered 94:358–359

Halterman D, Guenthner J, Collinge S, Butler N, Douches D (2016) Biotech potatoes in the 21st century: 20 years since the first biotech potato. Amer J Potato Res 93:1–20

Hamalainen JH, Watanabe KN, Valkonen JPT, Arihara A, Plaisted RL et al (1997) Mapping and marker assisted selection for a gene for extreme resistance to potato virus Y. Theor Appl Genet 94:192–197

Hamilton JP, Hansey CN, Whitty BR, Stoffel K, Massa AN et al (2011) Single nucleotide polymorphism discovery in elite North American potato germplasm. BMC Genom 12:302

Hancock RD, Morris WL, Ducreux LJ, Morris JA, Usman M, Verrall SR, Fuller J, Simpson CG, Zhang R, Hedley PE, Taylor MA (2014) Physiological, biochemical and molecular responses of the potato (*Solanum tuberosum* L.) plant to moderately elevated temperature. Plant, Cell Environ 37:439–450

Hane DC, Hamm PB (1999) Effects of seedborne potato virus Y infection in two potato cultivars expressing mild disease symptoms. Plant Dis 83:43–45

Hawkes JG (1994) Origins of cultivated potatoes and species relationships. In: Bradshaw JE, Mackay GR (eds) Potato Genetics. CAB International, Wallingford, pp 3–42

Hendriks T, Vreugdenhil D, Stiekema WJ (1991) Patatin and four serine proteinase inhibitor genes are differentially expressed during potato tuber development. Plant Mol Biol 17:385

Hofvander P, Ischebeck T, Turesson H, Kushwaha SK, Feussner I et al (2016) Potato tuber expression of Arabidopsis WRINKLED1 increase triacylglycerol and membrane lipids while affecting central carbohydrate metabolism. Plant Biotechnol J 14:1883–1898

Hosaka K, Hosaka Y, Mori M, Maida T, Matsunaga H (2001) Detection of a simplex RAPD marker linked to resistance to potato virus Y in a tetraploid potato. Amer J Potato Res 78:191–196

Hsieh TH, Lee JT, Charng YY, Chan MT (2002) Tomato plants ectopically expressing *Arabidopsis* CBF1 show enhanced resistance to water deficit stress. Plant Phyisol 130:618–626

Hu Y, Jiang L, Wang F, Yu D (2013) Jasmonate regulates the inducer of CBF expression-c-repeat binding factor/DRE binding Factor1 cascade and freezing tolerance in Arabidopsis. Plant Cell 25:2907–2924

Huang S, Weigel D, Beachy RN, Li J (2016) A proposed regulatory framework for genome-edited crops. Nat Genet 48:109–111

IPCC (2007) Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the IPCC. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M et al (eds) Cambridge. Cambridge University Press, UK, p 996

Itkin M, Rogachev I, Alkan N, Rosenberg T, Malitsky S et al (2011) GLYCOALKALOID METABOLISM1 is required for steroidal alkaloid glycosylation and prevention of phytotoxicity in tomato. Plant Cell 23:4507–4525

Jaccoud D, Peng KM, Feinstein D, Kilian A (2001) Diversity Arrays: a solid state technology for sequence information independent genotyping. Nucleic Acids Res 29:e25

Jacobs JME, van Eck HJ, Arens P, Verkerk-Bakker B, Te Lintel Hekkert B et al (1995) A genetic map of potato (*Solanum tuberosum*) integrating molecular markers, including transposons, and classical markers. Theor Appl Genet 91:289–300

Jacobs JME, van Eck HJ, Horsman K, Arens PFP, Verkerk-Bakker B et al (1996) Mapping of resistance to the potato cyst nematode *Globodera rostochiensis* from the wild potato species *Solanum vernei*. Mol Breed 2:51–60

Jacobs MM, Vosman B, Vleeshouwers VG, Visser RG, Henken B, van den Berg RG (2010) A novel approach to locate *Phytophthora infestans* resistance genes on the potato genetic map. Theor Appl Genet 120:785–796

James C (2010) Global Status of Commercialized Biotech/GM Crops: 2010. In: ISAAA Brief No 42. Ithaca, NY

Jansky SH, Simon R, Spooner DM (2009) A test of taxonomic predictivity: resistance to the Colorado potato beetle in wild relatives of cultivated potato. J Econ Entomol 102:422–431

Jo KR, Kim CJ, Kim SJ, Kim TY, Bergervoet M et al (2014) Development of late blight resistant potatoes by cisgene stacking. BMC Biotechnol 14:50

Kamthan A, Chaudhuri A, Kamthan M, Datta A (2016) Genetically modified (GM) crops: milestones and new advances in crop improvement. Theor Appl Genet 129:1639–1655

Kant S, Bi YM, Rothstein SJ (2011) Understanding plant response to nitrogen limitation for the improvement of crop nitrogen use efficiency. J Exp Bot 62:1499–1509

Kaplan F, Kopka J, Haskell DW, Zhao W, Schiller KC, Gatzke N, Sung DY, Guy CL (2004) Exploring the temperature-stress metabolome of Arabidopsis. Plant Physiol 136:4159–4168

Kasai K, Morikawa Y, Sorri VA, Valkonen JPT, Gebhardt C, Watanabe KN (2000) Development of SCAR markers to the PVY resistance gene *Ryadg* based on a common feature of plant disease resistance genes. Genome 43:1–8

Khan MA, Gemenet DC, Villordon A (2016) Root system architecture and abiotic stress tolerance: current knowledge in root and tuber crops. Front Plant Sci 7:1584

Kim-Lee H, Moon JS, Hong YJ, Kim MS, Cho HM (2005) Bacterial wilt resistance in the progenies of the fusion hybrids between haploid of potato and *Solanum commersonii*. Amer J Potato Res 82:129–137

Kloosterman B, Abelenda JA, Gomez Mdel M, Oortwijn M, de Boer JM et al (2013) Naturally occurring allele diversity allows potato cultivation in northern latitudes. Nature 495:246–250

Kloosterman B, Vorst O, Hall RD, Visser RG, Bachem CW (2005) Tuber on a chip: differential gene expression during potato tuber development. Plant Biotechnol J 3:505–519

Krauss A, Marschner H (1984) Growth rate and carbohydrate metabolism of potato tubers exposed to high temperatures. Potato Res 27:297–303

Krunic SL, Skryhan K, Mikkelsen L, Ruzanski C, Shaik SS et al (2018) Non-GMO potato lines with an altered starch biosynthesis pathway confer increased-amylose and resistant starch properties. Starch 70:1600310

Kuhl JC, Hanneman RE, Havey MJ (2001) Characterization and mapping of *Rpi*1, a late blight resistance locus from diploid (1EBN) Mexican *Solanum pinnatisectum*. Mol Genet Genom 265:977–985

Kumar P, Jander G (2017) Concurrent overexpression of *Arabidopsis thaliana* cystathionine gamma-synthase and silencing of endogenous methionine gamma-lyase enhance tuber methionine content in Solanum tuberosum. J Agri Food Chem 65:2737–2742

Lammerts van Bueren E, Verhoog H, Tiemens-Hulscher M, Struik P, Haring M (2007) Organic agriculture requires process rather than product evaluation of novel breeding techniques. NJAS Wageningen J Life Sci 54:401–412

Larkin PJ, Scowcroft WR (1981) Somaclonal variation-a novel source of variability from cell cultures for plant improvement. Theor Appl Genet 60:197–214

Lehsten V, Wiik L, Hannukkala A, Andreasson E, Chen D, Ou T, et al (2017) Earlier occurrence and increased explanatory power of climate for the first incidence of potato late blight caused by *Phytophthora infestans* in Fennoscandia. PLoS One 12(5): e0177580. Leonards-Schippers C, Gieffers W, Schafer-Pregl R, Ritter E, Knapp SJ et al (1994) Quantitative resistance to *Phytophthora infestans* in potato: a case study for QTL mapping in an allogamous plant species. Genetics 137: 67–77

Levy D, Veilleux RE (2007) Adaptation of potato to high temperatures and salinity-a review. Amer J Potato Res 84:487–506

Li L, Tacke E, Hofferbert HR, Lubeck J, Strahwald J et al (2013) Validation of candidate gene markers for marker-assisted selection of potato cultivars with improved tuber quality. Theor Appl Genet 126:1039–1052

Li L, Yang Y, Xu Q, Owsiany K, Welsch R et al (2012) The Or gene enhances carotenoid accumulation and stability during post-harvest storage of potato tubers. Mol Plant 5:339–352

Li Y, Tang W, Chen J, Jia R, Ma L et al (2016) Development of marker-free transgenic potato tubers enriched in caffeoylquinic acids and flavonols. J Agri Food Chem 64:2932–2940

Lindhout P, Meijer D, Schotte T, Hutten RC, Visser RG, Van Eck J (2011) Towards F_1 hybrid seed potato breeding. Potato Res 54:301–312

Liu F, Jensen CR, Shahanzari A, Andersen MN, Jacobsen SE (2005). ABA regulated stomatal control and photosynthetic water use efficiency of potato (*Solanum tuberosum* L.) during progressive soil drying. Plant Sci 168(3):831–836

Liu Z, Halterman D (2006) Identification and characterization of RB-orthologous genes from the late blight resistant wild potato species *Solanum verrucosum*. Physiol Mol Plant Pathol 69:230–239

Lokossou AA, Park TH, van Arkel G, Arens M, Ruyter-Spira C et al (2009) Exploiting knowledge of R/Avr genes to rapidly clone a new LZ-NBS-LRR family of late blight resistance genes from potato linkage group IV. Mol Plant Microbe Interact 22:630–641

Lozano R, Ponce O, Ramirez M, Mostajo N, Orjeda G (2012) Genome-wide identification and mapping of NBS-encoding resistance genes in *Solanum tuberosum* group Phureja. PLoS ONE 7:e34775

Luo ZW, Hackett CA, Bradshaw JE, McNicol JW, Milbourne D (2001) Construction of a genetic linkage map in tetraploid species using molecular markers. Genetics 157:1369–1385

Lynch JP (2007) Roots of the second green revolution. Aust J Bot 55:493–512

Machida-Hirano R (2015) Diversity of potato genetic resources. Breed Sci 65:26–40

Machida-Hirano R, Niino T (2017) Potato genetic resources. In: Chakrabarti SK, Xie C, Tiwari JK (eds) The Potato Genome. Springer, Switzerland, pp 11–30

Mackay I, Powell W (2007) Methods for linkage disequilibrium mapping in crops. Trends Plant Sci 12:57–63

Martinez CA, Maestri M, Lani EG (1996) In vitro salt tolerance and proline accumulation in Andean potato (*Solanum* spp) differing in frost resistance. Plant Sci 116:177–184

Martinez-Garcia J, Virgos-Soler A, Prat S (2002) Control of photoperiod-regulated tuberization in potato by the Arabidopsis flowering-time gene CONSTANS. Proc Natl Acad Sci USA 99:15211–15216

Massa AN, Childs KL, Buell CR (2013) Abiotic and biotic stress responses in group Phureja DM1-3 516 R44 as measured through whole transcriptome sequencing. Plant Genome 6:15

Massa AN, Childs KL, Lin H, Bryan GJ, Giuliano G, Buell CR (2011) The transcriptome of the reference potato genome *Solanum tuberosum* Group Phureja clone DM1-3 516R44. PLoS ONE 6:e26801

McCord P, Zhang LH, Brown C (2012) The incidence and effect on total tuber carotenoids of a recessive zeaxanthin epoxidase allele (Zep1) in yellow-fleshed potatoes. Amer J Potato Res 89:262–268

Milczarek D, Flis B, Przetakiewicz A (2011) Suitability of molecular markers for selection of potatoes resistant to *Globodera* spp. Amer J Potato Res 88:245–255

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7:405–410

Moloney C, Griffin D, Jones PW, Bryan GJ, McLean K et al (2010) Development of diagnostic markers for use in breeding potatoes resistant to *Globodera pallida* pathotype Pa2/3 using germplasm derived from *Solanum tuberosum* ssp *andigena* CPC 2802. Theor Appl Genet 120:679–689

Monneveux P, Ramírez DA, Khan MA, Raymundo RM, Loayza H, Quiroz R (2014) Drought and heat tolerance evaluation in potato (*Solanum tuberosum* L.). Potato Res 57:225–247

Monte MN, Rey Burusco MF, Carboni MF, Castellote MA, Sucar S, Norero NS, Colman SL, Massa GA, Colavita ML, Feingold SE (2018) Genetic diversity in Argentine Andean Potatoes by means of functional markers. Amer J Potato Res. 95(3):286–300

Morrell PL, Buckler ES, Ross-Ibarra J (2012) Crop genomics: advances and applications. Nat Rev Genet 13:85–96

Mustonen L, Wallius E, Hurme T (2008) Nitrogen fertilization and yield formation of potato during a short growing period. Agri Food Sci 9:173–183

Myles S, Peiffer J, Brown PJ, Ersoz ES, Zhang ZW et al (2009) Association mapping: critical considerations shift from genotyping to experimental design. Plant Cell 21:2194–2202

Naimov S, Dukiandjiev S, de Maagd RA (2003) A hybrid *Bacillus thuringiensis* delta-endotoxin gives resistance against a coleopteran and a epidopteran pest in transgenic potato. Plant Biotechnol J 1:51–57

Navarro C, Abelenda JA, Cruz-Oro E, Cuellar CA, Tamaki S, Silva J, Shimamoto K, Prat S (2011) Control of flowering and storage organ formation in potato by Flowering Locus T. Nature 478:119–122

Papp I, Mur LA, Dalmadi A, Dulai S, Koncz C (2004) A mutation in the Cap Binding Protein 20 gene confers drought tolerance to Arabidopsis. Plant Mol Biol 55(5):679–686

Park TH, Gros J, Sikkema A, Vleeshouwers VG, Muskens M et al (2005) The late blight resistance locus *Rpi-bib*3 from *Solanum bulbocastanum* belongs to a major late blight R gene cluster on chromosome 4 of potato. Mol Plant Microbe Interact 18:722–729

Pehu E, Gibson RW, Jones MGK, Karp A (1990) Studies on the genetic basis of resistance to potato leaf roll virus, potato virus Y and potato virus X in *Solanum brevidens* using somatic hybrids of *Solanum brevidens* and *Solanum tuberosum*. Plant Sci 69:95–101

Pel MA, Foster SJ, Park T-H, Rietman H, Arkel G et al (2009) Mapping and cloning of late blight resistance genes from *Solanum venturii* using an interspecific candidate gene approach. Mol Plant Microbe Interact 22:601–615

Pelletier Y, Horgan FG, Pompon J (2013) Potato resistance against insect herbivores: Resources and opportunities. In: Giordanengo P, Vincent C, Alyokhin A (eds) Insect Pests of Potato, Global Perspectives on Biology and Management. Academic Press, Oxford, UK, pp 439–462

Potato Genome Sequencing Consortium (2011) Genome sequence and analysis of the tuber crop potato. Nature 475:189–195

Prasad PVD, Potluri SDP (1996) Influence of proline and hydroxyproline on salt-stressed axillary bud cultures of two varieties of potato (*Solanum tuberosum*). Vitro Cell Devel Biol Plant 32:47–50

Pruvot G, Massimino J, Peltier G, Rey P (1996) Effects of low temperature, high salinity and exogenous ABA on the synthesis of two chloroplastic drought-induced proteins in *Solanum tuberosum*. Physiol Plant 97:123–131

Queitsch C, Hong S, Vierling E, Lindquist S (2000) Heat shock protein 101 plays a crucial role in thermotolerance in Arabidopsis. Plant Cell 12:479–492

Rahnama H, Ebrahimzadeh H (2005) The effect of NaCl on antioxidant enzyme activities in potato seedlings. Biol Plant 49:93–97

Rauscher GM, Smart CD, Simko I, Bonierbale M, Mayton H et al (2006) Characterization and mapping of *Rpi-ber*, a novel potato late blight resistance gene from *Solanum berthaultii*. Theor Appl Genet 112:674–687

Raymundo R, Asseng S, Robertson R, Petsakos A, Hoogenboom G et al (2018) Climate change impact on global potato production. Eur J Agron 100:87–98

Reynolds MP, Ewing EE (1989) Effects of high air and soil temperature stress on growth and tuberization in *Solanum tuberosum*. Ann Bot 64:241–247

Richardson KVA, Wetten AC, Caligari PDS (2001) Cell and nuclear degradation in root meristems following exposure of potatoes (*Solanum tuberosum* L.) to salinity. Potato Res 44:389–399

Ricroch AE, Hénard-Damave M-C (2015) Next biotech plants: new traits, crops, developers and technologies for addressing global challenges. Crit Rev Biotechnol 8551:1–16

Rykaczewska K (2013) The impact of high temperature during growing season on potato cultivars with different response to environmental stresses. Amer J Plant Sci 4:2386–2393

Ryu SB, Costa A, Xin ZG, PH Li (1995) Induction of cold hardiness by salt stress involves synthesis of cold responsive and abscisic acid responsive proteins in potato (*Solanum commersonii* Dun.). Plant Cell Physio 1(36): 1245–1251

Sabbah S, Tal M (1995) Salt tolerance in *Solanum kurzianum* and *S. tuberosum* cvs Alpha and Russet Burbank. Potato Res 38:319–330

Sanghera GS, Wani SH, Hussain W, Singh NB (2011) Engineering cold stress tolerance in crop plants. Curr Genom 12:30–43

Sarkar D, Tiwari JK, Sharma SU, Poonam Sharma SA et al (2011) Production and characterization of somatic hybrids between *Solanum tuberosum* L. and *S. pinnatisectum* Dun. Plant Cell Tiss Org Cult 107:427–440

Sattelmacher B, Klotz F, Marschner H (1990) Influence of the nitrogen level on root growth and morphology of two potato varieties differing in nitrogen acquisition. Plant Soil 132:131–137

Scheben A, Wolter F, Batley J, Puchta H, Edwards D (2017) Towards CRISPR/Cas crops- bringing together genomics and genome editing. New Phytol 216:682–698

Shinozaki K, Yamaguchi-Shinozaki K (2000) Molecular responses to dehydration and low temperature: Differences and cross-talk between two stress signaling pathways. Curr Opin Plant Biol 3:217–223

Silva JAB, Otoni WC, Martinez CA, Dias LM, Silva MAP (2001) Microtuberization of Andean potato species (*Solanum* spp.) as affected by salinity. Sci Hort 89:91–101

Singh BP, Dua VK, Govindakrishnan PM, Sharma S (2013) Impact of climate change on potato. In: Singh HP, Rao NKS, Shivashankara KS (eds) Climate-Resilient Horticulture: Adaptation and Mitigation Strategies. Springer, India, pp 125–136

Sliwka J, Jakuczun H, Chmielarz M, Hara-Skrzypiec A, Tomczynska I et al (2012) A resistance gene against potato late blight originating from *Solanum michoacanum* maps to potato chromosome VII. Theor Appl Genet 124:397–406

Śliwka J, Jakuczun H, Lebecka R, Marczewski W, Gebhardt C, Zimnoch-Guzowska E (2006) The novel, major locus *Rpi-phu*1 for late blight resistance maps to potato chromosome IX and is not correlated with long vegetation period. Theor Appl Genet 113:685–695

Smilde WD, Brigneti G, Jagger L, Perkins S, Jones JD (2005) *Solanum mochiquense* chromosome IX carries a novel late blight resistance gene *Rpi-moc*1. Theor Appl Genet 110:252–258

Smith S, De Smet I (2012) Root system architecture: insights from Arabidopsis and cereal crops. Philos Trans Roy Soc B Biol Sci 367:1441–1452

Song J, Bradeen JM, Naess SK, Raasch JA, Wielgus SM et al (2003) Gene RB cloned from *Solanum bulbocastanum* confers broad spectrum resistance to potato late blight. Proc Natl Acad Sci USA 100:9128–9133

Szajko K, Strzelczyk-Zyta D, Marczewski W (2014) Ny-1 and Ny-2 genes conferring hypersensitive response to potato virus Y (PVY) in cultivated potatoes: mapping and marker-assisted selection validation for PVY resistance in potato breeding. Mol Breed 34:267–271

Takahashi D, Li B, Nakayama T, Kawamura Y, Uemura M (2013) Plant plasma membrane proteomics for improving cold tolerance. Front Plant Sci 4:90

Tambussi EA, Bort J, Guiamet JJ, Araus JL (2007) The photosynthetic role of ears in C3 cereals: metabolism water use efficiency and contribution to grain yield. Crit Review Plant Sci 26:1–16

Tan MYA, Hutten RCB, Celis C, Park TH, Niks RE et al (2008) The *Rpi-mcd*1 locus from *Solanum microdontum* involved in resistance to *Phytophthora infestans*, causing a delay in infection, maps on potato chromosome 4 in a cluster of NBS-LRR genes. Mol Plant Microbe Interact 21:909–918

Tester M, Langridge P (2010) Breeding technologies to increase crop production in a changing world. Science 327:818–822

Tiwari JK, Ali N, Devi S, Kumar V, Zinta R, Chakrabarti SK (2018a) Development of microsatellite markers set for identification of Indian potato varieties. Sci Hort 231:22–30

Tiwari JK, Chandel P, Singh BP, Bhardwaj V (2014) Analysis of plastome and chondriome genome types in potato somatic hybrids from *Solanum tuberosum* x *Solanum etuberosum*. Genome 57:29–35

Tiwari JK, Devi S, Ali N, Luthra SK, Kumar V et al (2018b) Progress in somatic hybridization research in potato during the past 40 years. Plant Cell Tiss Org Cult 132:225–238

Tiwari JK, Devi S, Buckesth T, Ali N, Singh RK et al (2019) Precision phenotyping of contrasting potato (*Solanum tuberosum* L.) varieties in a novel aeroponics system for improving nitrogen use efficiency: in search of key traits and genes. J Integr Agri 18:2–12

Tiwari JK, Devi S, Sundaresha S, Chandel P, Ali N et al (2015) Microarray analysis of gene expression patterns in the leaf during potato tuberization in the potato somatic hybrid *Solanum tuberosum* and *Solanum etuberosum*. Genome 58:305–313

Tiwari JK, Gopal J, Singh BP (2012) Marker-assisted selection for virus resistance in potato: options and challenges. Potato J 39:101–117

Tiwari JK, Plett D, Garnett T, Chakrabarti SK, Singh RK (2018) Integrated genomics, physiology and breeding approaches for improving nitrogen use efficiency in potato: translating knowledge from other crops. Funct Plant Biol 45: 587–605

Tiwari JK, Poonam Sarkar D, Pandey SK, Gopal J, Kumar SR (2010) Molecular and morphological characterization of somatic hybrids between *Solanum tuberosum* L. and *S. etuberosum* Lindl. Plant Cell Tiss Org Cult 103:175–187

Tiwari JK, Sundaresha S, Singh BP, Kaushik SK, Chakrabarti SK et al (2013) Molecular markers for late blight resistance breeding of potato: an update. Plant Breed 132:237–245

Tuberosa R, Giuliani S, Parry MAJ, Araus JL (2007) Improving water use efficiency in Mediterranean agriculture: what limits the adoption of new technologies? Ann Appl Biol 150:157–162

Uitdewilligen JGAML, Wolters AMA, D'hoop BB, Borm TJA, Visser RGF, van Eck HJ (2013) A next-generation sequencing method for genotyping-by-sequencing of highly heterozygous autotetraploid potato. PLoS One 8: e62355

Upadhyaya CP, Akula N, Young KE, Chun SC, Kim DH, Park SW (2010) Enhanced ascorbic acid accumulation in transgenic potato confers tolerance to various abiotic stresses. Biotechnol Lett 32:321–330

van der Vossen E, Sikkema A, Hekkert BL, Gros J, Stevens P et al (2003) An ancient R gene from the wild potato species *Solanum bulbocastanum* confers broad-spectrum resistance to *Phytophthora infestans* in cultivated potato and tomato. Plant J 36:867–882

van der Vossen EAG, Gros J, Sikkema A, Muskens M, Wouters D et al (2005) The *Rpi-blb*2 gene from *Solanum bulbocastanum* is an Mi-1 gene homolog conferring broad-spectrum late blight resistance in potato. Plant J 44:208–222

Van Eck J, Conlin B, Garvin D, Mason H, Navarre D, Brown C (2007) Enhancing beta-carotene content in potato by RNAi-mediated silencing of the beta-carotene hydroxylase gene. Amer J Potato Res 84:331–342

Velásquez B, Balzarini M, Taleisnik E (2005) Salt tolerance variability amongst Argentine Andean potatoes (*Solanum tuberosum* L subsp. *andigena*). Potato Res 48:59–67

Vierling E (1991) The roles of heat-shock proteins in plants. Annu Rev Plant Physiol 42:579–620

Villamon FG, Spooner DM, Orrillo M, Mihovilovich E, Pérez W, Bonierbale M (2005) Late blight resistance linkages in a novel cross of the wild potato species *Solanum paucissectum* (series Piurana). Theor Appl Genet 111:1201–1214

Vleeshouwers VGAA, Rietman H, Krenek P, Champouret N, Young C et al (2008) Effector genomics accelerates discovery and functional profiling of potato disease resistance and *Phytophthora infestans* avirulence genes. PLoS ONE 3:e2875

Voorrips RE, Gort G, Vosman B (2011) Genotype calling in tetraploid species from bi-allelic marker data using mixture models. BMC Bioinformatics 12:172

Vos J (1999) Split nitrogen application in potato: effects on accumulation of nitrogen and dry matter in the crop and on the soil nitrogen budget. J Agri Sci Camb 133:263–274

Vos P, Hogers R, Bleeker M, Reijans M, Vandelee T et al (1995) AFLP: a new technique for DNA-fingerprinting. Nucleic Acids Res 23:4407–4414

Wang M, Allefs S, Berg R, Vleeshouwers VGAA, Vossen EAG, Vosman B (2008) Allele mining in Solanum: conserved homologues of *Rpi-blb*1 are identified in *Solanum stoloniferum*. Theor Appl Genet 116:933–943

Watanabe KN, Kikuchi A, Shimazaki T, Asahina M (2011) Salt and drought stress tolerances in transgenic potatoes and wild species. Potato Res 54:319–324

Werij JS, Furrer H, van Eck HJ, Visser RGF, Bachem CWB (2012) A limited set of starch related genes explain several interrelated traits in potato. Euphytica 186:501–516

Wishart J, George TS, Brown LK, Ramsay G, Bradshaw JE et al (2013) Measuring variation in potato roots in both field and glasshouse: the search for useful yield predictors and a simple screen for root traits. Plant Soil 368:231–249

Wittwer CT, Reed GH, Gundry CN, Vandersteen JG, Pryor RJ (2003) High-resolution genotyping by amplicon melting analysis using LCGreen. Clin Chem 49:853–860

Xie Z, Khanna K, Ruan S (2010) Expression of microRNAs and its regulation in plants. Semin Cell Dev Biol 21(8):790–797

Yan H, Ito H, Nobuta K, Ouyang S, Jin W et al (2006) Genomic and genetic characterization of rice Cen3 reveals extensive transcription and evolutionary implications of a complex centromere. Plant Cell 18:2123–2133

Ye M, Peng Z, Tang D, Yang Z, Li D et al (2018) Generation of self-compatible diploid potato by knockout of S-RNase. Nat Plants 4:651–654

Zaheer K, Akhtar MH (2016) Potato production, usage, and nutrition–a review. Crit Rev Food Sci Nutr 56:711–721

Zeigler RS, Mohanty S (2010) Support for international agricultural research: current status and future challenges. New Biotechnol 27:566–572

Zhang HX, Blumwald E (2001) Transgenic salt-tolerant tomato plants accumulate salt in foliage but not in fruit. Nat Biotechnol 19:765–768

Zhang HX, Hodson JN, W'flliams JP, Blumwald E (2001) Engineering salt-tolerant *Brassica* plants: Characterization of' yield and seed oil quality in transgenic plants with increased vacuolar sodium accumulation. Proc Natl Acad Sci USA 98:12832–12836

Zhang ZJ, Li HZ, Zhou WJ, Takeuchi Y, Yoneyama K (2006) Effect of 5-aminolevulinic acid on development and salt tolerance of potato (*Solanum tuberosum* L.) microtubers in vitro. Plant Growth Regul 49:27–34

Zhang ZJ, Mao BZ, Li HZ, Zhou WJ, Takeuchi Y, Yoneyama K (2005) Effect of salinity on physiological characteristics, yield and quality of microtubers in vitro in potato. Acta Physiol Plant 27:481–489

Zhu J, Brown KM, Lynch JP (2010) Root cortical aerenchyma improves the drought tolerance of maize (*Zea mays* L.). Plant, Cell Environ 33:740–749

Zhu X, Gong H, He Q, Zeng Z, Busse JS, Jin W, Bethke PC, Jiang J (2016) Silencing of vacuolar invertase and asparagine synthetase genes and its impact on acrylamide formation of fried potato products. Plant Biotechnol J 14(2):709–718

Zimnoch-Guzowska E, Marczewski W, Lebecka R, Flis B, Schafer-Pregl R et al (2000) QTL analysis of new sources of resistance to *Erwinia carotovora* ssp. *atroseptica* in potato done by AFLP, RFLP, and resistance-gene-like markers. Crop Sci 40:1156–1167

Chapter 2
Genomic Designing for Climate-Smart Tomato

Mathilde Causse, Jiantao Zhao, Isidore Diouf, Jiaojiao Wang, Veronique Lefebvre, Bernard Caromel, Michel Génard and Nadia Bertin

Abstract Tomato is the first vegetable consumed in the world. It is grown in very different conditions and areas, mainly in field for processing tomatoes while fresh-market tomatoes are often produced in greenhouses. Tomato faces many environmental stresses, both biotic and abiotic. Today many new genomic resources are available allowing an acceleration of the genetic progress. In this chapter, we will first present the main challenges to breed climate-smart tomatoes. The breeding objectives relative to productivity, fruit quality, and adaptation to environmental stresses will be presented with a special focus on how climate change is impacting these objectives. In the second part, the genetic and genomic resources available will be presented. Then, traditional and molecular breeding techniques will be discussed. A special focus will then be presented on ecophysiological modeling, which could constitute an important strategy to define new ideotypes adapted to breeding objectives. Finally, we will illustrate how new biotechnological tools are implemented and could be used to breed climate-smart tomatoes.

Keywords Tomato · Breeding · Productivity · Biotic stress · Abiotic stress · Ideotypes · Modeling

M. Causse (✉) · J. Zhao · I. Diouf · V. Lefebvre · B. Caromel
INRA, Centre de Recherche PACA, Génétique et Amélioration Des Fruits et Légumes, Domaine Saint Maurice, CS60094, Montfavet 84143, France
e-mail: mathilde.causse@inrae.fr

J. Wang
INRA and University of Bordeaux, UMR 1332 Biologie Du Fruit et Pathologie, 71, Av. Edouard Bourlaux - CS, 20032-33882 Villenave D'Ornon Cedex, France

M. Génard · N. Bertin
INRA, Plantes et Systèmes de Culture Horticoles, Institut National de La Recherche Agronomique - Centre de Recherche PACA, Avignon, France

C. Kole (ed.), *Genomic Designing of Climate-Smart Vegetable Crops*,
https://doi.org/10.1007/978-3-319-97415-6_2

2.1 Introduction

Tomato is the first vegetable consumed worldwide following potato. It has become an important food in many countries. Two main types of tomato varieties are produced, tomatoes for the processing industry, with determinate growth produced only in open field and indeterminate growth varieties for fresh market, which may be grown in very diverse conditions, from open field to greenhouses with controlled conditions.

Tomato, *Solanum lycopersicum* L., is a member of the large Solanaceae family, together with potato, eggplant, and pepper. It is a self-pollinated crop, with a diploid ($2n = 2x = 24$) genome of medium size (950 Mb). A high-quality reference genome sequence was published in 2012 (The Tomato Genome Consortium 2012). Tomato originates from South America along with 12 wild relative species, which can be crossed with the cultivated tomato species. Several large collections of genetic resources exist and more than 70,000 varieties are conserved in these gene banks. The collections also include scientific resources such as collections of mutants or segregating populations.

Tomato is also a model species for genetic analysis since a long time. Many mutations inducing important phenotype variations were discovered and positionally cloned and many disease resistance genes were functionally characterized. Tomato is also a model species for fruit development and physiology. It is easy to transform and it has been the first transgenic food produced and sold (Kramer and Redenbaugh 1994).

In this chapter, we will first present the main challenges to breed climate-smart tomatoes. The breeding objectives relative to productivity, fruit quality, and adaptation to environmental stresses will be presented with a special focus on how climate change is impacting these objectives. In the second part, the genetic and genomic resources available will be presented. Then, traditional and molecular breeding techniques will be discussed. A special focus will then be presented on ecophysiological modeling, which could constitute an important strategy to define new ideotypes adapted to breeding objectives. Finally, we will illustrate how new biotechnological tools are implemented and could be used to breed climate-smart tomatoes.

2.2 Challenges, Priorities, and Breeding Objectives

Tomato crop faces several challenges, which impact its breeding objectives. Breeders will orient their main breeding objectives according to the wide diversity of growth conditions and use them as fresh or processed. These objectives can be classified into (1) productivity, (2) adaptation to growth conditions in terms of response to biotic and abiotic stresses, and (3) fruit quality at both nutritional and sensory levels.

2.2.1 Productivity

From 1988 to 2017, the tomato world production regularly grew from 64 to 182 MT. Since 1995, China increased its production and became the first producer, and since then, its production increased up to 60 MT (Fig. 2.1) covering almost 4,800,000 ha. This growth is due to an increase in the production area, but also due to improvement in productivity and variety breeding.

With an average yield of 37 T/ha, compared to 16 T/ha in 1961, the yield has increased over years but large differences remain according to countries and growth conditions. In south European greenhouses, the average yield is 50–80 T/ha, while it may be more than 400 T/ha in the Netherlands and Belgium, with a crop lasting up to 11 months. Expressed per square meter, the average yield is 3.7 kg/m^2, reaching 50 kg/m^2 in the Netherlands, while it is 5.6 in China where most of the production is in the open field although modern Chinese solar greenhouses are developed (Cao et al. 2019).

Tomato yield is strongly dependent on cultivars and growth conditions. Yield results from fruit number and fruit weight. Cultivars for fresh market are classified based on their fruit size and shape from the cherry tomato (less than 20 g) to beef tomato (fruit weight higher than 200 g). The potential size depends on cell number established in pre-anthesis stage, but the final fruit size mainly depends on the rate and duration of cell enlargement (Ho 1996). Seed number and competition among fruits also affect the final fruit size (Bertin et al. 2002, 2003). Seed and fruit are highly sensitive to biotic and abiotic stresses, which often lead to seed and fruit abortion (Ruan et al. 2012). Fruit number is controlled by the truss architecture but the increase in flower number often leads to abortion (Soyk et al. 2017a, b). Fruit shape varies from flat to long or ovate and is also determined at the carpel development stage.

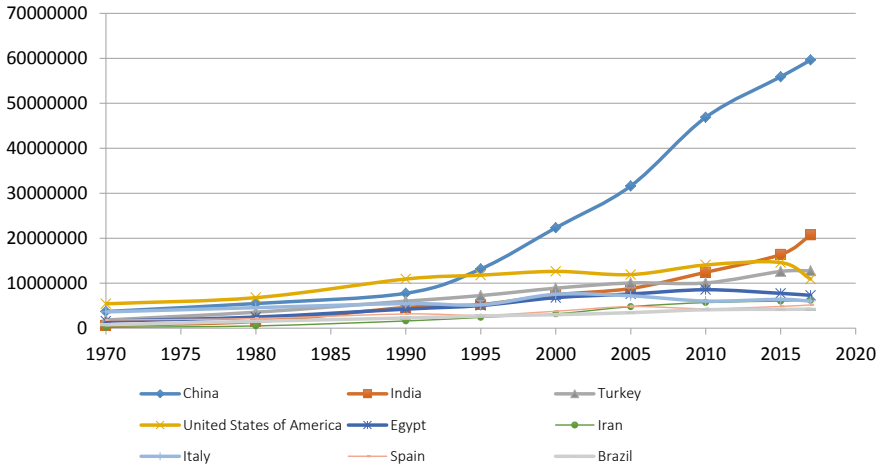

Fig. 2.1 Evolution of tomato production over years in the nine main producing countries

Mutations in four genes explain most of the tomato fruit shape (Rodríguez et al. 2011).

2.2.2 Fruit Quality

2.2.2.1 Nutritional Quality

Tomato consumption has been shown to reduce the risks of certain cancers and cardiovascular diseases (Giovannucci 1999). Its nutritional value is related to fruit composition in primary and secondary metabolites (Table 2.1) but is mostly due to its content in lycopene and carotene (Bramley 2000). Lycopene is responsible for the red fruit color but also acts as a dietary antioxidant. Tomato also constitutes an important source of vitamin C. In spite of considerable efforts in developing cultivars with higher content of carotenoids, or vitamin C, none has reached a commercial

Table 2.1 Average tomato fruit nutritional value and composition (adapted from USDA)

Proximates	Content (per 100 g fresh weight)
Water	94.5 g
Energy	18 kcal
Protein	0.88 g
Lipids	0.2 g
Fibers	1.2 g
Sugars	2.63 g
Acids	0.65 g
Minerals	
Calcium	10 mg
Magnesium	11 mg
Phosphorus	24 mg
Potassium	237 mg
Sodium	5 mg
Fluoride	g
Vitamins	
Vitamin C	14 mg
Choline	6.7 mg
Vitamin A and carotene	0.59 mg
Lycopene	2.57 mg
Lutein and zeaxanthin	123 g
Vitamin K	8 g

(adapted from USDA: https://www.usda.gov/)

importance, in part because of a negative correlation between yield and these traits (Klee 2010).

In addition to these well-known vitamins and antioxidants, other compounds in tomato fruit with antioxidant properties include chlorogenic acid, rutin, plastoquinones, tocopherol, and xanthophylls. Tomatoes also contribute but to a lesser extent to carbohydrates, fiber, flavor compounds, minerals, protein, fats, and glycoalkaloids to the diet (Davies and Hobson 1981). Exhaustive metabolome studies have described the composition of tomato in terms of both primary and secondary metabolites and has shown the wide diversity present among tomato accessions and their wild relatives (Tikunov et al. 2005; Schauer et al. 2006; Rambla et al. 2014; Wells et al. 2013; Tieman et al. 2017; Zhu et al. 2018).

Considerable genetic variation exists in tomato for micronutrients with antioxidant activity or other health-promoting properties (Hanson et al. 2004; Schauer et al. 2005). A number of these micronutrients, particularly carotenoids, have long been the major objectives of breeding programs because of their contribution to the quality of fresh and processed tomato products. Increased recognition of their health-promoting properties has stimulated new research to identify loci that influence their concentration in tomato.

Vitamin A and vitamin C are the principal vitamins in tomato fruit. Tomatoes also provide moderate levels of folate and potassium in the diet and lesser amounts of vitamin E and several water-soluble vitamins. Carotene biosynthesis in tomato has been deciphered and many genes and mutations have been identified (Ronen et al. 1999). More than 20 genes that influence the type, amount, or distribution of fruit carotenoids have been characterized in tomato (Labate et al. 2007).

Vitamin C pathway in plants has been deciphered by Smirnoff and Wheeler (2000). The variation in ascorbic acid content may depend on varieties and growth conditions (Gest et al. 2013) and a few quantitative trait loci (QTLs) controlling its variation have been identified (Stevens et al. 2007). The synthesis pathway of folate is also well characterized and the genes involved were identified (Almeida et al. 2011). One of the major QTLs controlling its variation has been shown to be related to an epigenetic variation (Quadrana et al. 2014).

Glycoalkaloids and their toxic effects are commonly associated with the Solanaceous species. Tomato accumulates the glycoalkaloids α-tomatine and dehydrotomatine which are less toxic than glycoalkaloids in potato (Madhavi and Salunkhe 1998; Milner et al. 2011). Several genes controlling their variations have been identified (Cárdenas et al. 2016; Zhu et al. 2018).

Tomato mineral composition is greatly influenced by plant nutrition (see below), and as a result, has been well characterized in the context of mineral deficiency and the effect of these conditions on plant health. There is a significant genotypic variation for mineral content in tomato fruit. Potassium, together with nitrate and phosphorous, constitutes approximately 93% of the total inorganic fruit constituents (Davies and Hobson 1981).

Flavonoids comprise a large group of secondary plant metabolites and include anthocyanins, flavonols, flavones, catechins, and flavonones (Harborne 1994). Numerous efforts have focused on the manipulation of transgene expression to

enhance fruit flavonoids (Muir et al. 2001; Bovy et al. 2002; Colliver et al. 2002). Willits et al. (2005) identified a wild accession that expressed structural genes of the anthocyanin biosynthetic pathway in the fruit peel and fruit flesh. Introgression of the *S. pennellii* accession into tomato produced progeny that accumulated high levels of quercetin in fruit flesh and peel. The mutation responsible for the lack of accumulation of yellow color flavonoid in the pink tomato has been identified (Adato et al. 2009; Ballester et al. 2016). Phenolic acids form a diverse group. Hydroxycinnamic acid esters of caffeic acid predominate in Solanaceous species and chlorogenic acid is the most abundant (Molgaard and Ravn 1988). Rousseaux et al. (2005) noted large environmental interactions for fruit antioxidants and identified several QTLs for total phenolic concentration in fruit of *S. pennellii* introgression lines.

2.2.2.2 Sensory Quality

Fresh-market tomato breeders improved yield, disease resistance, adaptation to greenhouse conditions, fruit aspect, but have lacked clear targets for improving organoleptic fruit quality. Consumers have complained about tomato taste for years (Bruhn et al. 1991). Nevertheless improving sensory fruit quality is complex as it is determined by a set of attributes, describing external (size, color, firmness) and internal (flavor, aroma, texture) properties.

Flavor is mostly due to sugars and organic acids (Stevens et al. 1977), to their ratio (Stevens et al. 1979; Bucheli et al. 1999), and to the composition in volatile aromas (Klee and Tieman 2013). Sweetness and acidity are related to the content of sugars and acids (Janse and Schols 1995; Malundo et al. 1995). Sweetness seems to be more influenced by the content in fructose than in glucose, while acidity is mostly due to the citric acid, present in higher content than malic acid in mature fruits (Stevens et al. 1977). Depending on the studies, acidity is more related to the fruit pH or to the titratable acidity (Baldwin et al. 1998; Auerswald et al. 1999). Both sugars and acids contribute to the sweetness and to the overall aroma intensity (Baldwin et al. 1998). More than 400 volatiles have been identified (Petró-Turza 1986), a few of them contributing to the particular aroma of tomato fruit (Baldwin et al. 2000; Tieman et al. 2017). Texture traits are more difficult to relate to physical measures or to fruit composition, although firmness in the mouth is partly related to the instrumental measure of fruit firmness (Causse et al. 2002), and mealiness was found related to the texture parameters of the pericarp (Verkerke et al. 1998). Several studies intended to identify the most important characteristics of consumer preferences (Causse et al. 2010).

Although production of high-quality fruits is dependent on environmental factors (light and climate) and cultural practices, a large range of genetic variation has been shown, which could be used for breeding tomato quality as reviewed by Davies and Hobson (1981), Stevens (1986), and Dorais et al. (2001). Causse et al. (2003) showed the importance of flavor and secondarily of texture traits in consumer appreciation. Cherry tomatoes have been identified as a source of flavor (Hobson and Bedford 1989), with fruits rich in acids and sugars. Long shelf life cultivars

have been described as generally less tasty than traditional ones (Jones 1986), with lower volatile content (Baldwin et al. 1991). Furthermore quality has a subjective component and there is not a unique expectation (Causse et al. 2010).

Wild relatives of *S. lycopersicum* may be an interesting source for improving fruit composition. Mutations of enzymes involved in the carbon metabolism were found in *S. chmielewskii* and in *S. habrochaites*, leading to particular sugar compositions: The *sucr* mutation in an invertase gene, in *S. chmielewskii,* provides fruits with sucrose instead of glucose and fructose (Chetelat et al. 1995). In *S. habrochaites*, an allele of the ADP glucose pyrophosphorylase enzyme was identified as much more efficient than the allele of the cultivated species, leading to an increase in the final sugar content of the fruit (Schaffer et al. 2000). Another locus *Fgr* modulates the fructose to glucose ratio in mature fruit, for which an allele from *S. habrochaites* yields higher fructose to glucose ratio (Levin et al.2000). The gene responsible is a sugar transporter of the SWEET family (Shammai et al. 2018). A gene *Lin5* encoding apoplastic invertase has been shown to be a QTL modulating sugar partitioning, the allele of *S. pennellii* leading to higher sugar concentrations than the *S. lycopersicum*one (Fridman et al. 2000). Wild tomato species may also provide original aromas, either favorable to tomato quality (Kamal et al. 2001) or unfavorable (Tadmor et al. 2002). Several genes responsible for the variation of aroma production in tomato have been cloned (Klee 2010; Bauchet et al. 2017a, b; Zhu et al. 2019).

Many efforts for improving fruit quality have failed because of the complex correlations between the various components or between yield or fruit weight and fruit components. The correlation between fruit weight and sugar content is frequently negative (Causse et al. 2001), but may be positive in other samples (Grandillo and Tanksley 1996a). In several studies involving sensory evaluation and fruit composition analyses, sweetness was positively correlated with reducing sugar content and sourness with titratable acidity (Baldwin et al. 1998; Causse et al. 2002). The firm texture is positively correlated with the instrumental firmness (Lee et al. 1999; Causse et al. 2002). Correlations were also detected between fruit size and antioxidant composition (Hanson et al. 2004). High-throughput metabolic profiling allowed getting insight on the whole metabolic changes in tomato fruits during fruit development or in various genotypes (Schauer et al. 2005; Overy et al. 2004; Baxter et al. 2007).

Addressing the demand of the producers and retailers of fresh-market tomatoes, breeders have considerably improved the external aspect and shelf life of tomato fruit. This improvement was obtained either by the use of ripening mutations or by the cumulative effect of several genes improving fruit firmness. Several mutations affecting fruit ripening are known, *rin* (ripening inhibitor) the most widely used, *nor* (non-ripening), and *alc* (alcobaca). Long shelf life cultivars have entered into the tomato market in the 1990s, but consumers have criticized their flavor (Jones 1986; McGlasson et al. 1987). The corresponding genes have been identified and extensively studied (Vrebalov et al. 2002; Ito et al. 2017; Wang et al. 2019). The impact of the enzymes involved in cell wall modifications during ripening on fruit firmness and shelf life has been extensively studied and modifications of polygalacturonase or pectin methylesterase activity were proposed to increase fruit shelf life and texture properties (Hobson and Grierson 1993).

Processing tomato has specific quality attributes. The self-pruning mutation (*sp*), characteristic of all the processing varieties, controls the determinate growth habit of tomato plants. Processing cultivars associate the *sp* mutation with concentrated flowering, fruit firmness, and resistance of mature fruits to overripening, allowing a unique mechanical harvest. The *sp* gene was cloned (Pnueli et al. 1998). This mutation does not only affect plant architecture, but also modulates the expression of genes controlling fruit weight and composition (Stevens 1986; Fridman et al. 2002; Quinet et al. 2011). This gene belongs to a gene family that is composed of at least six genes (Carmel-Goren et al. 2003). Recently, *sp* gene was also shown to be responsible for the loss of day-length-sensitive flowering (Soyk et al. 2017a, b). The jointless mutations, provided by the *j* and *j2* genes, are also useful for processing tomato production. The *j2* mutation has been discovered in a *S. cheesmaniae* accession, and has no abscission zone in fruit pedicel allowing harvest without calyx and pedicel during vine pick-up (Mao et al. 2000; Budiman et al. 2004).

2.2.2.3 Mild Stress as a Tool to Manage Quality

Tomatoes are produced all year round under contrasting environmental conditions, triggering seasonal variations in their sensory quality. Over the tomato growth cycle, different factors such as light intensity, air and soil temperatures, plant fruit load, plant mineral nutrition, or water availability influence the final fruit quality (reviewed in Davies and Hobson 1981; Poiroux-Gonord et al. 2010). Variations in temperature and irradiance during ripening affect carotene, ascorbic acid, and phenolic compound content in the fruit, although acid and sugar content are not modified considerably by these two factors (Venter et al. 1977; Rosales et al. 2007; Gautier et al. 2008). Changes in plant fruit load through trust pruning modify fruit dry matter content and final fruit fresh weight by disrupting the carbon flux entering the fruit (Bertin et al. 2000; Guichard et al. 2005). Water limitation and irrigation with saline water may positively impact tomato fruit quality, mainly through an increase in sugar content in fruit (either by concentration or accumulation effect) and contrasted effects on the secondary metabolite contents (Mitchell et al. 1991; De Pascale et al. 2001; Nuruddin et al. 2003; Johnstone et al. 2005; Gautier et al. 2008; Ripoll et al. 2016). The effects reported on fruit composition are associated or not with large yield loss depending upon the intensity and duration of the treatment and the development stage of the plant (Ripoll et al. 2014; Guichard et al. 2001; Albacete et al. 2015; Osorio et al. 2014).

Thus, the optimization of the growth practice, in particular, water management, is considered in horticultural production as a tool to manage fruit quality while limiting yield losses, offering the opportunity to address simultaneously environmental issues and consumer expectations of tastier fruits (Stikic et al. 2003; Fereres and Soriano 2006; Costa et al. 2007). The genetic variability of tomato response to water limitations and other abiotic constraints and their combination still need to be deciphered to develop genotypes adapted to these practices (Poiroux-Gonord et al. 2010; Ripoll et al. 2014). Large phenotypic variation in response to a wide range of climate and

nutrition conditions exists in the genus *Solanum* at both inter- and intraspecies levels (reviewed in Labate et al. 2007).

Several authors attempted to measure genotype-by-environment (GxE) interactions on tomato fruit quality by repeating the same experiment in different locations or/and under several growing facilities (Auerswald et al. 1999; Johansson et al. 1999; Causse et al. 2003) or by building experimental design to isolate the effect of particular environmental factors on large number of genotypes (see Semel et al. 2007; Gur et al. 2011; Albert et al. 2016a; for water availability and Monforte et al. 1996, 1997a, b for salt stress). In different experiments, the G x E interaction was significant for the fruit quality traits measured (including fruit fresh weight, secondary and primary metabolism contents, and fruit firmness), but generally accounted for a low part of the total variation in comparison to the genotype main effect. Albert et al. (2016a) dissected further the genotype by watering regime interaction in an intraspecific *S. lycopersicum* recombinant inbred line population grown under two contrasting watering regimes in two locations. Besides, they detected large genetic variation and genetic heritabilities under both watering regimes, encouraging the possibility to develop tomato genotypes with an improved fruit quality under mild water stress.

2.2.3 Biotic and Abiotic Stresses

2.2.3.1 Biotic Stresses

Pests and Pathogens of Tomatoes

Pests and pathogens cause great damage to tomato crops in field and in greenhouse. Tomato is afflicted by at least 200 pests and pathogens, from most major classes such as bacteria, fungi, oomycetes, viruses, nematodes, insects, and spider mites (Foolad and Panthee 2012). Insects are as diverse as aphids, thrips, whiteflies, leafminers, fruit borers, caterpillars, leafhoppers; they disturb the foliage development perturbing photosynthesis carbon assimilation, deform fruit appearance, and ultimately reduce the yield. Moreover several of them may transmit viruses. A few viruses may also be transmitted by contact such as Tobamoviruses. Foolad and Panthee (2012) made a compendium of the most important diseases on tomato caused by 21 fungi, 1 oomycete, 7 bacteria, 7 viruses, and 4 nematodes.

Diseases contribute to almost 40% of tomato yield loss in the field worldwide. The occurrence of those diseases varies according to the geographical regions where tomatoes are grown, environmental conditions, and cultural practices. For instance, high relative humidity favors the stem canker and the early blight caused by different species of *Alternaria*, and warm air temperature and damp conditions favor the gray leaf spot caused by different species of *Stemphylium* while low soil temperature favors the corky root rot caused by *Pyrenochaeta lycopersici* and cool air temperature favors the *Fusarium* crown and root rot. Otherwise, high air humidity alternating with

cool night temperature is favorable for the development of late blight caused by the Oomycete *Phytophthora infestans* that can easily destroy up to 100% of field or greenhouse tomato crops.

Impact of Climate Change on Pest and Pathogen Resistance

Climatic prediction models indicate severe weather pattern changes, which will result in frequent droughts and floods, rising global temperatures, and decreased availability of fresh water for agriculture. A great challenge is thus to improve the robustness of plant resistance and tolerance to pests and pathogens, to a wide array of combined biotic and abiotic stress combinations. Tomato crops are exposed to multiple abiotic stresses in fields and greenhouses that could attenuate or enhance the response to biotic stress. Recent studies have revealed that the response of plants to combinations of two or more stress conditions is unique and cannot be directly extrapolated from the response of plants to each stress applied individually. Few studies report the tomato responses to biotic x abiotic stress combinations.

It is well known for a long time that high temperatures (above 30 °C) inhibit plant defense mechanisms making major resistance genes frequently dysfunctional. For instance, the tomato *Mi-1.2* resistance gene to root knot nematode and *Cf-4*/*Cf-9* genes to *Cladosporium fulvum* are inactivated at high temperature (de Jong et al. 2002; Marques de Carvalho et al. 2015). Other abiotic stresses could also modify tomato immunity. For instance, drought stress reduces disease severity to *Botrytis cinerea* and stops the development of *Oidium neolycopersici*. Irrigation with saline water increases disease severity to *Fusarium oxysporum f. sp. radicis-lycopersici* and to *Phytophthora capsici*, does not affect *Botrytiscinerea* infection, and reduces infection by *O. neolycopersici* (Achuo et al. 2006; Dileo et al. 2010). Bai et al. (2018) suggest that salt stress modifies the hormone balance involved in sthe ignaling pathway that could decrease the resistance level conferred by the *Ol-1* gene but has no effect on resistance conferred by *Ol-2* and *Ol-4* genes, those three genes controlling *O. neolycopersici* responsible for tomato powdery mildew. Limited nitrogen or water supplies increase tomato stem susceptibility to *B. cinerea* (Lecompte et al. 2017). Very high environmental pressure caused by elevated ozone concentration eliminates the effect of potato spindle tuber viroid (PSTVd) on biomass reduction in tomato (Abraitiene and Girgzdiene 2013). The few examples cited here mainly focused on the effect of environmental changes on tomato immunity controlled by major resistance genes. Much less publications concern resistance QTLs yet, even if research on the effect of G x E interactions on resistance to biotic stress is increasing. Actually, there is a knowledge gap in the identification of QTLs involved in responses to combined biotic antibiotic stresses.

New Emerging Tomato Diseases

Global climate change is supposed to result in the emergence of new pests and pathogens into production areas. Tomato health management is thus challenged by the emergence of new races that overcome resistance genes deployed in cultivars and by novel introductions due to the world's agricultural market and the climate change. Several diseases are reemerging or emerging on tomato crops such as the late blight caused by *P. infestans* (Fry and Goodwin 1997), the leafminer *Tuta absoluta*, and new viruses that increasingly affect tomato crops. The Potexvirus *Pepino mosaic virus* (PepMV), mainly mechanically transmitted, emerged around 2000 and causes now significant problems on glasshouse tomato crops worldwide (Hanssen and Thomma 2010). Recently, the *tomato brown rugose fruit virus* (ToBRFV), a new tobamovirus present in Jordania and Israel, was able to break *Tm-2*-mediated resistance in tomato that had lasted 55 years (Maayan et al. 2018). The emergence of new viruses is often coupled with the proliferation of adapting insect vectors. Tomato production in tropical countries is severely constrained by insects and mites, particularly whiteflies (*Bemisia tabaci*) that could transmit begomoviruses (including TYLCV known for a long time but also many other emergent begomoviruses) and fruit borers that cause serious problems during the reproductive phase of the crop. Deploying host resistance against viruses, when available, is actually the most effective method for controlling viruses and preventing their spread, even if in recent years resistance-breaking strains of viruses have been characterized, against which these resistance genes are no longer effective. For example, the resistance gene *Sw-5* confers resistance to TSWV transmitted by the thrips *Frankliniella occidentalis*, as well as to related orthotospovirus species such as *Groundnut ring spot virus* (GRSV) and *Tomato chlorotic spot virus* (TCSV) recently emerged in the United States and the Caribbean. But it has been overcome by new virulent TSWV strains (Oliver and Whitfield 2016; Turina et al. 2016).

In addition, the bacteria *Clavibacter michiganense* subsp. *michiganensis* (Cmm), causing the bacterial canker disease devastating tomato production worldwide, is considered as a real plague. This bacteria is one of the few pathogens transmitted by seeds. To fight the spread of this disease, Good Seed and Plant Practices (GSPP; https://www.gspp.eu/), adopted by sites or companies working on tomato breeding and plantlet production, prevent tomato seed and plant lots from being infected by Cmm. GSPP-accredited sites or companies are granted the right to market their tomato seeds and young plants with the GSPP logo. The first GSPP seed and plants have been available since July 2011 in France and the Netherlands.

So far, there is no sufficiently sustainable or effective genetic leverage available for tomato breeding programs to combat these new diseases. Their sustainable control is a goal of global importance, which will probably require combining several genetic strategies associated with cultural practices to effectively manage those novel pathosystems.

2.2.3.2 Abiotic Stresses

Tomato domestication and improvement have focused for a long time on agronomic traits associated with productivity, quality, and disease resistance. Crop resilience facing the global climate change nowadays represents one of the most challenging aspects of plant breeding, raising awareness in developing climate-smart crops. It has led to the characterization of new breeding traits related to abiotic stress tolerance. Understanding the complex genetic architecture of plant response to environmental changes appears to be central for the development of new cultivars. Indeed, variations in environmental factors usually induce some disorders at molecular, physiological, and morphological levels that may alter the agronomic performance of crops. Stress adaptation in plants at the molecular level requires generally the activation of multiple stress-response genes that are involved in different metabolic pathways for growth maintenance and which expression is regulated by various transcription factors (TFs). The genomic era facilitated the characterization of such stress-response genes across plant species that were assigned to a diverse family of TFs. The major families of TFs playing significant roles in stress tolerance that were described in the literature include the basic leucine zipper (bZIP), dehydration-responsive element binding protein (DREB), APETALA 2 and ethylene-responsive element binding factor (AP2/ERF), zinc fingers (ZFs), basic helix-loop-helix (bHLH), heat-shock proteins (Hsp), and others (Lindemose et al. 2013). The functions covered by these TFs are very common in the plant kingdom; however, each species presents specificities.

In tomato, Bai et al. (2018) characterized the 83 WRKY genes identified in previous studies and displayed their different roles in response to pathogen infection, drought, salt, heat, and cold stresses. Some genes were highlighted as being altered in their expression by different stress such as drought and salinity stress (*SlWRKY3; SlWRKY3, and SlWRKY33*) pointing pertinent candidates for further investigation. The expression profiles of other tomato stress-response genes were also investigated for a class of genes belonging to the ERFs family (Klay et al. 2018) and Hsp20 gene family (Yu et al. 2017). Examples of single genes involved in tomato tolerance to abiotic stress were also described including the *SlJUB1* promoting drought tolerance; *DREB1A* and *VP1.1* playing a role in salinity tolerance, and *ShDHN, MYB49,* and *SlWRKY39* for tolerance to multi-stress factors (Liu et al. 2015; Sun et al. 2015; Cui et al. 2018).

Tomato is a suitable plant model to study the genetics of plant response to the environment and for deciphering the genotype-by-interaction (GxE) mechanisms, due to the wide range of environmental conditions—from fields to greenhouse cultivation—for its production highlighting its large adaptability.

Water Deficit

Tomato is a high water-demanding crop (Heuvelink 2005) making water resource management one of the key factors essential for the crop. The amount of irrigation

water in tomato production is usually managed according to the reference evapotranspiration (ET_0) and the developmental stage. When water deficit (WD) occurs during the cropping period, morphological and molecular changes are usually observed that hamper the final yield production. Several studies addressed the impact of WD stress on tomato, most of which establishing WD as a percentage of water restriction, according to the optimal water requirement (Albert et al. 2016a, b; Ripoll et al. 2016; Diouf et al. 2018).

From an agronomic point of view, the main consequence of WD on tomato is yield reduction that can be severe when stress occurs during fruit development (Chen et al. 2013). However, all developmental stages are susceptible to WD to a level depending on the cultivar and stress intensity. Seed germination is the first step exposed to environmental stress. In tomato, a delay or even an inhibition of seed germination was observed with the application of osmotic stress (Bhatt and Rao 1987). Water deficit during vegetative and reproductive development negatively affects the overall economic performance of the crop but positive effects on fruit quality are documented. Indeed, Costa et al. (2007) described some trade-off between yield decrease and increase in quality component on fruit trees and vegetables including tomato where enhancement in fruit quality compounds such as vitamin C, antioxidants, and soluble sugars was observed under WD stress (Albert et al. 2016a; Ripoll et al. 2014; Patanè and Cosentino 2010; Zegbe-Domínguez et al. 2003). The two groups of accessions constituted of cherry tomato and large fruit accessions usually show different sensitivity to environmental stresses. For instance, a study using a panel of unrelated lines tested under control and WD conditions revealed that large fruit tomato accessions were more susceptible and had higher responsiveness to WD (Albert et al. 2016b). This study also showed that the increase in the sugar content in fruit under WD is due to a reduction in fruit water content and not due to increased synthesis of sugars. However, Ripoll et al. (2016) found higher fructose and glucose synthesis in tomato fruits submitted to WD stress for different stages of fruit development, indicating that both dilution effect and higher sugar synthesis are responsible for fruit quality enhancement in tomato under WD. The omics approaches allow targeting specific genes and studying their variation in expression level according to different environmental conditions. Some examples of water deficit response genes involved in tomato tolerance to drought are published. This is the case for *SlSHN1* gene that induces tolerance to drought by activating downstream genes involved in higher cuticular wax accumulation on leaves (Al-Abdallat et al. 2014). Tolerance to drought induces early activation of signaling pathways to elicit drought-related genes. Wang et al. (2018) identified a drought-induced gene (*SlMAPK1*) playing an active role in the antioxidant enzyme activities and ROS scavenging leading to higher drought tolerance.

Salinity Stress

Soil salinity has become problematic in agriculture especially in the Mediterranean region where soil aridification and non-sustainable irrigation practices tend

to increase the surface area of salty soils (Munns and Tester 2008). Munns and Gilliham (2015) defined salinity stress (SS) as the level of salinity up to which the energy for plant growth is redirected into defense response. Considering yield as a measure of tolerance to SS, tomato is a crop that can tolerate up to 2.5 dS m^{-1} of salinity and cherry tomatoes are less salt sensitive than large fruit accessions (Scholberg and Locascio 1999; Caro et al. 1991). Over the above-mentioned threshold, a significant yield decrease is observed. Yield reduction under SS in tomato was found to be associated with a reduction in both fruit size and fruit number (Scholberg and Locascio 1999). As for WD, SS also leads to an increase in sugar content in tomato fruits (Mitchell et al. 1991). Besides, SS leads to changes in the cation/anion ratio and the increase in sugar content in fruits of salinized plants likely results from the interaction between reduced fruit water content, increased ion content, and maintained hexose accumulation (Navarro et al. 2005). These changes are the consequences of tomato response to the osmotic adjustment. The threshold for salinity tolerance defined above was set upon the characterization of a few selected tomato cultivars. However, Alian et al. (2000) noticed a high genotypic variability in response to salinity in fresh-market tomato cultivars. This highlights the possibility and the potentiality for the crop to breed salt-tolerant cultivars.

Facing SS, plants deploy a variety of response to rebalance and reestablish the cellular homeostasis. Physiological responses to SS involve the ionic channels transporters as they are highly needed to regulate the ionic imbalance (Apse et al. 1999). In their study, Rajasekaran et al. (2000) screened salinity tolerance in a number of tomato wild relatives and associated salinity tolerance mainly to a higher K^+/NA^+ ratio in roots. High genetic variability was observed in *S. pimpinellifolium* accessions for yield and survival traits in response to SS (Rao et al. 2013). Among yield component traits, fruit number was the most affected trait in both wild and cultivated populations (Rao et al. 2013; Diouf et al. 2018). Breeding salt-tolerant variety thus seems possible by using either physiological traits or agronomic performance under salinity, as sufficient genetic variability is available in several tomato genetic resources.

Temperature Stress

All crop species have an optimal temperature range for growth. Tomato is known as a crop that can grow in a wide range of environments, from elevated areas with low temperatures to tropical and arid zones where high temperatures usually occur. Based on the crop simulation model, Boote et al. (2012) indicated that the optimal growth for tomato and its fruit development is about 25 °C. Temperatures below 6 °C and above 30 °C severely limit growth, pollination, and fruit development and could negatively impact final fruit yield. Studies on different accessions and wild relative species of tomato helped understanding how the crop responds to low and high-temperature stresses.

High-temperature stress

The most visible effect of climate change is the rise in temperature in different areas of the world. The end of the twenty-first century is expected to come with the increase in global warming causing significant yield decrease in major worldwide cultivated crops (Zhao et al. 2017). When plants are exposed to fluctuating high temperatures (HT), ensuing stress is considered as short-term heat stress; when the period of exposure to HT is short or long-term heat stress. if plants experienced the HT for several consecutive days. The latter has more dramatic effects on agronomic performances of crops, especially when it occurs during the entire cropping season. In open field trials, seed germination is more generally impaired by high temperature of the soil and can differ to the effects of elevated air temperatures. However, flowering period is described as the most critical stage under HT stress (Wahid et al. 2007). Severe yield decrease caused by HT stress arises from the hampered reproduction performance with a high impact of HT on reproductive organs (Nadeem et al. 2018). In tomato, HT stress around flowering was shown to inhibit reproduction by altering male fertility at a high degree and female fertility at a lower rate (Xu et al. 2017a, b). In areas where the temperature range could be reliably predicted, managing the sowing date to avoid HT stress around anthesis is an important factor to consider. Tomato male fertility could be considered as the main factor limiting reproduction success under HT stress. This has led some studies to use pollen traits as a measure of heat tolerance instead of only final yield (Driedonks et al. 2018). Male reproductive traits were highly variable among wild species and some accessions showed high pollen viability compared to cultivated cultivars. This opens possibilities for transferring heat-tolerant alleles from wild donors to cultivated tomato. A reduction of fruit setting was also observed in cultivated tomato with a higher rate of parthenocarpic fruits noticed under HT stress at 26 °C in growth chambers (Adams et al. 2001). These authors noticed that fruit maturation is accelerated under higher temperature mostly when fruits are exposed themselves to heating periods, that could alter final fruit quality composition.

Considering the important effect of HT on agriculture, numerous studies successfully tackled and identified several heat-response genes (Waters et al. 2017; Keller and Simm 2018; Fragkostefanakis et al. 2016). Heat-response genes are commonly regulated by the activity of several heat stress transcription factors (HSFs) as described in the literature for different organisms. This has led to the investigation of the roles played by HSFs in thermo-tolerance and majors HSFs depicted across plant species could lead to the development of heat-tolerant tomato via genome editing(Fragkostefanakis et al. 2015).

Chilling and cold stress

Chilling stress (CS) is usually considered when plants are growing in temperature below the optimal growth range and above 0 °C, just before freezing stress. The geographical distribution of wild tomato species includes elevated zones where annual temperatures can be below the optimal growth for cultivated tomatoes (Nakazato et al. 2010). This denotes that adaptation to sub-optimal temperature is possible in tomato.

Adams et al. (2001) observed that at 14 °C, tomato growth was reduced. Lower temperatures equally induce some chilling stress symptoms as reviewed by Ploeg and Heuvelink (2005) who noticed that below 12 °C, almost no growth is observed for tomato. As for HT stress, fruit set is inhibited in tomato mainly due to poorer pollen viability. Reduction in the number of flowers, number of fruits, and final yield was observed with low temperature that also affects the partitioning of photosynthetic products (Meena et al. 2018). Indeed, photosynthesis is highly impacted during CS and several related physiological parameters are described. For example, the relative water content, chlorophyll fluorescence, and accumulation of phenolic compounds are associated to mechanisms inducing cold tolerance (Giroux and Filion 1992; Dong et al. 2019; Khan et al. 2015). By the way, Meena et al. (2018) showed that external application of phenolic compounds—notably salicylic acids—significantly increased tomato tolerance to CS. Low-temperature stress during plant growth and development adversely affects the fruit quality of tomato and reduces non-enzyme antioxidants such as lycopene, β-carotene, and α-tocopherol.

Transcriptome analysis depicted some genes responding to CS in tomato. For example, Zhuang et al. (2019) identified a cold response tomato gene (*SlWHY1*) whose expression is enhanced under 4 °C, playing a role in photosystem II protection and starch accumulation in chloroplast. For several plant species, signal transmission of CS involves the C-repeat binding factor (CBF) (Jha et al. 2017) leading to downstream activation of cold responsive genes for cold tolerance. Major types of CBF are known to regulate cold acclimation in tomato (Mboup et al. 2012). In a recent review, Kenchanmane Raju et al. (2018) showed that genes related to photosynthesis and chloroplast development were consistently repressed in response to low temperature and the most conserved set of genes up-regulated in response to low-temperature stress belonged to the CBFs, WRKYs, and AP2/EREBP transcription factors. These results highlighted some genes and family of transcription factors that could be targeted for breeding tomato adapted to low-temperature conditions.

Mineral Nutrition Deficiency

The positive effect of mineral nutrition on plant growth has long been recognized and mineral elements are usually classified as essential or non-essential; the latter being, however, beneficial for plant development (Marschner 1983). The macronutrients are mostly necessary to stimulate growth and nitrogen (N), potassium (K^+), and phosphorus (P) are among the most important in higher plants. Their use has a significant environmental cost and thus selection for reduced need of fertilizer could be useful for the production of smart crops.

Nitrogen

Nitrogen *(N)* is among the most important limiting nutrient for tomato development. Insufficient N nutrition can cause severe consequences to economically important traits. It was shown that N-deficiency negatively affects the number of fruits, fruit size, storage quality, color, and taste of tomato (Sainju et al. 2003). As evidenced by de

Groot et al. (2004) and Larbat et al. (2012), tomato growth rate is linearly correlated to N supply. Low N supply limits growth in leaves but promotes root development and this activity was mainly linked to variation in cytokinin concentration. An increase in accumulation of phenolic compounds is also a notable consequence of N-deficiency in tomato. Indeed, Larbat et al. (2012) found that sequential limitation of N nutrition resulted in an up-regulation of genes associated with phenolic biosynthetic pathway.

Oversupply of N above the required optimal level is usual in tomato cultivation due to its beneficial effects and the willing to avoid the negative effects of limited N; however, excess of N can overproduce vegetative growth at the expense of fruit development and rapid fruit maturation and inhibits root system development besides its negative effect on groundwater pollution (Du et al. 2018). This highlights the necessity to manage N nutrition in tomato cropping that can be achieved through a good characterization of genes involved in nitrogen use efficiency. Apart from genetic solutions to improve tolerance to N-deficiency, real-time greenhouse management technics are now available with the use of computational intelligence systems and definition of new stress tolerance traits like leaf reflectance as proposed by Elvanidi et al. (2018).

Phosphorus

Phosphorus *(P)* is usually present in the soil in a form that is not accessible for plants. Fertilization is thus required for major crops including tomato. Plant capacity to acquire P present in the soil is associated to root morphological changes and involves variation in plant-hormone levels. Early plant development is very sensitive to P nutrition and sub-optimal P supply in tomato can lead to impaired growth and plant development (Sainju et al. 2003; de Groot et al. 2004). Phosphate deficiency induces modification in root architecture morphology via increased auxin sensitivity leading to the activation of P transporter genes to remobilize P from lipids and nucleic acids (Schachtman and Shin 2007). Long-term adaptation to P starvation appears to be linked to reduced primary root growth at the expanse of lateral root growth that is promoted (Xu et al. 2012). Besides, the net-photosynthesis decreased in the leaves with reduced sucrose content after long exposure to P starvation, while the starch content increased. These authors also identified different genes responding to P starvation that belong to the 14-3-3 gene family encoding phosphoserine-binding proteins involved in protein–protein interactions.

In open field conditions, a larger root system development may be required for greater exploration and acquisition of P present in the soil. For greenhouse production where the P input can be managed, the need is more in the characterization of P-deficiency response genes and their correlation to morphological and physiological response for the development of cultivars with higher P-use efficiency.

Potassium

The importance of *Potassium* (K^+) in plant nutrition has been attested with its involvement in important physiological processes such as photosynthesis, osmoregulation, and ion homeostasis (Marschner 1983; Pettigrew 2008). Yield and quality are known to be impacted by the photosynthesis capacity of the plant and thus could be directly

linked to the K^+ concentration in plant organs. In tomato, positive effects of K^+ supply have been described for vigorous growth, early flowering, fruit number production, and higher rate of titratable acidity (Sainju et al. 2003). Increase in soluble solids, antioxidative capacity, and ascorbic acid were also observed in tomato fruits (Tavallali et al. 2018) with K^+ supply. Alternatively, deficiency in K^+ nutrition induced morphological injuries resulting in brown marginal scorching with interveinal chlorosis and yellowing of tomato leaves. Indeed, plants usually sense external changes in K^+ concentration leading to the activation of signal transduction to reestablish the ion homeostasis. Adaptation to low K^+ supply is achieved through different K^+ movement monitored by different K^+ transporters. The function and role of different transporter channels involved in K^+ movement in plants were described by Wang and Wu (2015) including the *HAK/KUP/KT* family of transporters seemingly crucial for K^+ transport. The transport of K^+ in plants is initiated in the roots and the major impact of K^+ deficiency is on root architecture (Zhao et al. 2018). Improving root system development could then directly alleviate the deleterious effect of K^+ deficiency.

Calcium

Calcium is an important ion involved in diverse metabolic processes central to plant growth and development (Bush 1995). Several reviews regarding the role of this macronutrient on plants pinpoint its involvement in the cell wall rigidity, cell membrane stability, the control of ion transport, and the signaling of abiotic stress (Hepler 2005; Hirschi 2004; Wilkins et al. 2016). Calcium deficiency is associated with changes in the cell ion homeostasis and had been related to nutritional imbalance incidence, among other problems in plants. The diminution of Ca^{2+} nutrition as well as environmental stimuli has been considered as leading changes in the cytosolic concentration of Ca^{2+} mediating some modifications in Ca^{2+} flux through transporter proteins in order to reestablish the ion homeostasis (Bush 1995). Besides, plant response to abiotic stresses is tightly linked to modification in Ca^{2+} homeostasis essential to signaling and subsequent plant tolerance deployment (Rengel 1992; Wilkins et al. 2016). In tomato, Ca^{2+} nutrition under salinity stress, for example, has been shown to alleviate the negative impact induced by salt toxicity on plant and fruit growth (Tuna et al. 2007). This was linked to Ca^{2+} use efficiency upon the availability of sufficient Ca^{2+} concentration in the plant. Calcium-use efficiency is an important characteristic for plant adaptation to environmental stress and this trait is genetically variable indicating the possibility for breeding cultivars with high potentiality of adaptation to low Ca^{2+} input (Li and Gabelman 1990). However, most tomato accessions are susceptible to Ca^{2+} deficiency and among the undesirable effects associated with this stress, a physiological disorder at the fruit named blossom-end rot (BER) has been noticed (Adams and Ho 1993). Other studies correlate BER incidence to differences in genotype capacity to limit oxidative stress by increasing the synthesis of antioxidant metabolites such as ascorbate (Rached et al. 2018) or genotype sensitivity to gibberellin (Gaion et al. 2019) suggesting a non-direct effect of Ca^{2+} depletion in the cells to induce BER symptoms. Moreover, through transcriptomic analyses, de Freitas et al. (2018) identified candidate genes inhibiting BER in tomato

that were mostly associated with resistance against oxidative stress. Tomato BER is thus a complex physiological disorder occurring from the impact of abiotic stresses, genetic, physiological, or agronomic factors with possible interaction between them (Hagassou et al. 2019). However, regarding the tight link between BER and the level of Ca^{2+} in tomato, the characterization of the channel gene families involved in regulation of Ca^{2+} homeostasis under different environmental stimuli could help to disentangle the underlying molecular mechanisms of the interaction between BER incidence and Ca^{2+} concentration.

2.2.3.3 Stress Combination

Plant responses to individual stress at a specific growth stage are well documented and avenues for crop breeding to enhance tolerance to a particular stress were provided. However, observations in the nature and in open field conditions clearly brought to light that stress combination is a common phenomenon, especially with the climate change that has an incidence of co-occurring of environmental stresses such as WD and HT stress. Climate change trend has also an impact on pathogen spreading and new disease appearance and distribution (Harvell et al. 2002). Different scenarios of biotic and abiotic stress combination are then expected to arise, according to the geographical regions and areas of crop cultivation. With different crop species exposed to different stress treatments, Suzuki et al. (2014) presented a stress matrix with the potential positive and negative effects of various patterns of stress combination. The global effect of combined stresses on yield, morphological, and physiological traits on plants can be highly different from those of a single stress. Thus the stress matrix proposed by Suzuki et al. (2014) would be highly useful if specified for tomato, to achieve a global view of how stress combinations could be managed in breeding programs.

Examples of studies conducted in tomato to assess the impact of combined stress on different traits are available in the literature. Zhou et al. (2017) showed that physiological and growth responses to the combined WD and HT stresses had a similar pattern across different cultivars but the response was different from the single heat response. Combination of HT stress and SS on tomato showed, however, less damage on growth than the application of SS alone (Rivero et al. 2014). Besides morphological changes, some studies conducted on the model species *Arabidopsis thaliana* demonstrated that variations in gene expression under stress combination are highly independent of variation induced by single stress application (Rasmussen et al. 2013).

In addition to the combination of different environmental stresses, simultaneous biotic and abiotic stresses, which are usually studied separately, are expected, especially in field conditions. Recently, studies were performed to fill the lack of knowledge about the genetic response to biotic and abiotic stress combination compared to a single stress effect. In tomato, Kissoudis et al. (2015) studied the combined effect of salinity and powdery mildew (*Oidium neolycopersici*) infection and found that salt stress increases the powdery mildew susceptibility in an introgression line

population. Anfoka et al. (2016) showed that long-term HT stress was accompanied with TYLCV accumulation in tomato reducing by the way the HT response efficiency. Some stress responses such as endogenous phytohormone secretion and ROS production are important physiological processes involved in both abiotic and biotic plant responses (Fujita et al. 2006) that could require the action of a group of genes regulating both types of stresses. Some genes were shown to be involved in the simultaneous response to biotic and abiotic stress on tomato such as the *SlGGP-LIKE* gene that Yang et al. (2017) found to be correlated to higher ascorbic acid synthesis, less ROS damage, and higher tolerance to chilling stress, however, its suppression led to higher ROS accumulation and resistance to *P. syringae*. Using genomic data from multiple stress-response genes, Ashrafi-Dehkordi et al. (2018) performed a comparative transcriptome analysis on tomato and found a set of genes the expression of which is altered under simultaneous biotic and abiotic stresses. Single tomato genes involved in responses to both abiotic stresses and *Pseudomonas syringae* (Sun et al. 2015) or *Phytophthora infestans* (Cui et al. 2018) were identified making them suitable targets for breeding. However, up to now, stress combination is mostly addressed in a genomic or metabolomics point of view and few examples of genetic response to combined stress are documented except in *A. thaliana* (Thoen et al. 2017).

The impact of mineral nutrition on plant pathogen is also important: the enhanced phenolic and volatile compounds accumulated with N fertilization have been shown to interact with tomato disease induced by insect attacks such as whitefly, *Bemisia tabaci* (Islam et al. 2017), and leafminer *Tuta absoluta* Han et al. (2015). Interaction between N supply and tomato resistance to *Botrytis cinerea* has also been described (Lecompte et al. 2010). Nitrogen supply not only interacts with biotic tolerance in tomato but has also a different impact according to some abiotic factors.

Among abiotic stresses, salinity is the most important stress in tomato affecting tomato responses. The simultaneous effect of salinity stress and N input was measured by Papadopoulos and Rendig (1983) who showed that the positive effects of N supply on growth and fruit weight were suppressed by salinity stress reaching up to 5 dS m^{-1}.

In an interspecific introgression line (IL) population, (Frary et al. 2011) showed that salinity decreased the leaf Ca^{2+} content by 47% and K^{+} content by 8%. *S. pennellii* alleles were found contributing mostly to higher Ca^{2+} content under both control and salinity stress suggesting this species as a natural resource for salinity and low Ca^{2+} input stress tolerance.

2.3 Genetic and Genomic Resources for Trait Breeding

2.3.1 Genetic Resources

2.3.1.1 Origin of Tomato and Its Wild Relatives

Genetic resources for food and agriculture are keys to global food security and nutrition (FAO 2015). In crop production, maintaining genetic diversity is an essential strategy not only to breed new varieties, to identify candidate genes of target traits, to dissect the evolutionary history, but also to reduce the effects of biotic and abiotic stresses, etc.

Tomato belongs to the large and diverse Solanaceae family also called Nightshades, which includes more than three thousand species. Among them, major crops arose from Old world (eggplant from Asia) and New world (pepper, potato, tobacco, tomato from South America). The *Lycopersicon* clade (Table 2.2) contains the domesticated tomato (*Solanum lycopersicum*) and its 12 closest wild relatives (Peralta et al. 2005). Charles Rick and colleagues started the first prospections and studies on the tomato wild relatives in the 1940s.

Tomato clade species are originated from the Andean region, including Peru, Bolivia, Ecuador, Colombia, and Chile. Their growing environments range from sea level to 3,300 m altitude, from arid to rainy climate and from Andean Highlands to the coast of Galapagos Islands. Their habitats are often narrow and isolated valleys and they were adapted to many climates and different soil types. The large range of ecological conditions contributed to the diversity of the wild species. This broad variation is also expressed at the morphological, physiological, sexual, and molecular levels (Peralta et al. 2005).

The domestication of tomato is due to a divergence from *S. pimpinellifolium* that occurred several thousand years ago. It probably happened in two steps, first in Peru, leading to *S. lycopersicum cerasiforme* accessions then in Mexico, leading to large fruit accessions (reviewed in Bauchet and Causse 2012) and confirmed by molecular analyses (Blanca et al. 2012; Lin et al. 2014; Blanca et al. 2015). Only a few tomato seeds were brought back from Mexico to Europe, leading, after domestication, to a new genetic bottleneck. The tomato cultivation first slowly spread in southern Europe and it is only after the Second World War that its intentional selection started and that it was spread over the world.

2.3.1.2 Genetic Resources as Sources for Adaptation

There are more than 83,000 tomato accessions stored in different seed banks worldwide (FAO 2015). These seed banks include the Tomato Genetic Resources Center (TGRC) in Davis, USA (https://tgrc.ucdavis.edu/), the United States Department of Agriculture (USDA) in Geneva, USA (https://www.ars.usda.gov/), the World Vegetable Center in Taiwan, (https://avrdc.org/), the Centre for Genetic

Table 2.2 Tomatoes and their wild relative species of the Lycopersicon section according to Peralta et al. 2005 "Lycopersicon group" corresponds to the red- and orange-fruited species). For further details of crossability and other biological parameters of wild tomatoes see Grandillo et al. (2011)

Species	Distribution	Habitat; (elevational range	Section according to Peralta et al. (2005)
Solanum lycopersicum L.	Globally cultivated domesticate	Cultivated; sea level-4000 m	*Lycopersicon* "Lycopersicon group"
Solanum pimpinellifolium L.	Southwestern Ecuador to northern Chile (many northern populations in Ecuador are admixture with *S. lycopersicum*; Peralta et al. 2005; Blanca et al. 2013)	Dry slopes, plains and around cultivated fields; sea level-3000 m	*Lycopersicon* "Lycopersicon group"
Solanum peruvianum L.	Central Peru to northern Chile	Dry coastal deserts and lomas; sea level-3000 m	*Lycopersicon* "Eriopersicon group"
Solanum cheesmaniae (L. Riley) Fosberg	Galápagos Islands	Dry, open, rocky slopes; sea level-1300 m	*Lycopersicon* "Lycopersicon group"
Solanum galapagense S.C. Darwin and Peralta	Galápagos Islands	Dry, open, rocky slopes; seashores; sea level-1600 m	*Lycopersicon* "Lycopersicon group"
Solanum arcanum Peralta	Northern Peru	Dry inter-Andean valleys and in coastal lomas (seasonal fog-drenched habitats); 100–4000 m	*Lycopersicon "Arcanum group"*
Solanum chmielewskii (C.M. Rick, Kesicki, Fobles & M. Holle) D.M. Spooner, G.J. Anderson & R.K. Jansen	Southern Peru and northern Bolivia	Dry inter-Andean valleys, usually on open, rocky slopes; often on roadcuts; 1200–3000 m	*Lycopersicon "Arcanum group"*
Solanum neorickii D.M. Spooner, G.J. Anderson & R.K. Jansen	Southern Ecuador to southern Peru	Dry inter-Andean valleys; 500–3500 m	*Lycopersicon "Arcanum group"*

(continued)

Table 2.2 (continued)

Species	Distribution	Habitat; (elevational range	Section according to Peralta et al. (2005)
Solanum chilense (Dunal)Reiche	Coastal Chile and southern Peru	Dry, open, rocky slopes; sea level-4000 m (B. Igic, pers. comm. Has suggested the higher elevation plants represent a new species)	*Lycopersicon "Eriopersicon group"*
Solanum corneliomulleri J.F. Macbr.	Southern Peru (Lima southwards)	Dry, rocky slopes; 20–4500 m (low elevation populations associated with landslides in southern Peru)	*Lycopersicon "Eriopersicon group"*
Solanum habrochaites S. Knapp and D.M. Spooner	Andean Ecuador and Peru	Montane forests, dry slopes and occasionally coastal lomas; 10–4100 m	*Lycopersicon "Eriopersicon group"*
Solanum huaylasense Peralta	Río Santa river drainage, north-central Peru	Dry, open, rocky slopes; 950–3300 m	*Lycopersicon "Eriopersicon group"*
Solanum pennellii Correll	Northern Peru to northern Chile	Dry slopes and washes, usually in flat areas; sea level-4100 m	*Lycopersicon "Neolycopersicon group"*

Resources, in the Netherlands (https://www.wur.nl/en/Research-Results/Statutory-research-tasks/Centre-for-Genetic-Resources-the-Netherlands-1.htm), and others. These seed banks maintain most of the genetic diversity of tomatoes.

Thanks to the pioneering work of Charles Rick, the Tomato Genetics Resource Center of the University of California, in Davis, maintains the largest collection of wild relative accessions that he prospected during his life. This collection has been an important source of diversity for breeding tomato and for gene discovery. For instance, there is a collection of 46 *S. pennellii* that is only found in Peru, and is particularly adapted to dry conditions (Fig. 2.2).

2.3.1.3 Natural and Induced Mutants

Natural genetic diversity is the main source of adaptation and crop breeding. Natural mutations appeared in cultivated accessions or were introduced from wild relative species, which provide a great source of genetic diversity for many traits, including disease resistance genes and quality trait-related genes (Bauchet and Causse 2012;

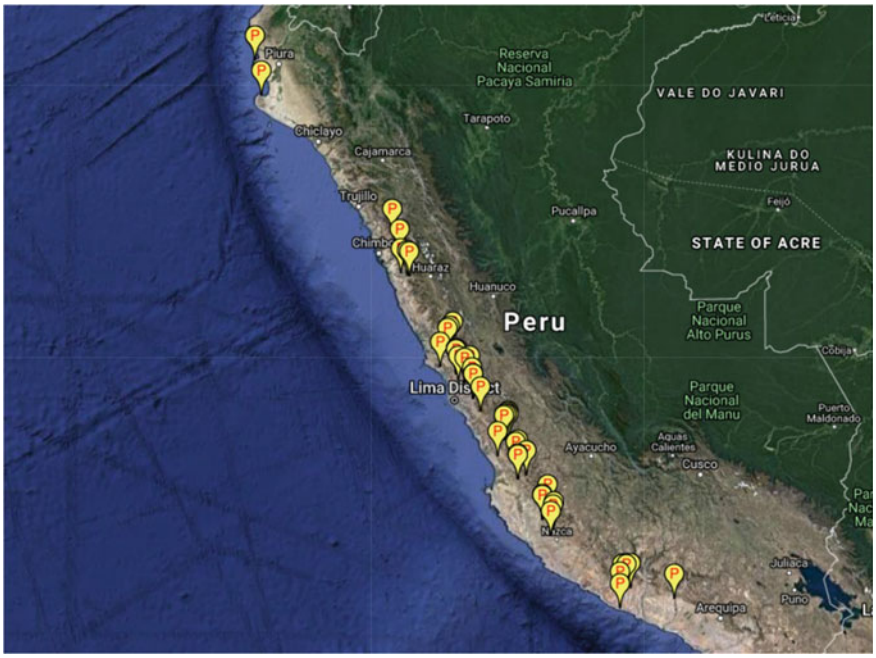

Fig. 2.2 Geographical locations of wild tomato species *Solanum pennellii*. Data were collected from Tomato Genetics Resource Center, University of California, Davis (https://tgrc.ucdavis.edu/Data/Acc/Wildspecies.aspx)

Bauchet et al. 2017a; Rothan et al. 2019). However, the number of cloned genes with detailed functional validations is still limited (Rothan et al. 2019). Some biotechnology tools such as TILLING (Targeting Induced Local Lesions in Genomes; Comai and Henikoff 2006) provide collections of mutants in a specific accession, accelerating functional genomic research and the discovery of interesting alleles at a given locus (Menda et al. 2004; Baldet et al. 2007; Okabe et al. 2011; Mazzucato et al. 2015; Gauffier et al. 2016). This technology typically uses chemical mutagens such as ethyl methanesulfonate (EMS) to generate several base mutations in the genome. There are several TILLING collections worldwide for tomato, such as the UCD Genome Center TILLING laboratory, University of California, USA (http://tilling.ucdavis.edu/index.php/TomatoTilling); The Microtom collection (Okabe et al. 2011); TOMATOMA database, Japan (http://tomatoma.nbrp.jp/); The Repository of Tomato Genomics Resources, University of Hyderabad, India (https://www.uohyd.ac.in/images/index.html); The Genes That Make Tomatoes (http://zamir.sgn.cornell.edu/mutants/index.html); the Tilling Platform of Tomato, INRA, France (http://www-urgv.versailles.inra.fr/tilling/tomato.htm) (Minoïa et al. 2010); LycoTILL database, Metapontum Agrobios, Italy (http://www.agrobios.it/tilling/) (Minoia et al. 2010) and others.

2.3.2 Molecular Markers and Gene/QTL Mapping

2.3.2.1 Evolution of Molecular Markers

Tomato has been used for genetic studies and mutation mapping of interesting traits even before the discovery of molecular markers (Butler 1952). Genes of interest were first mapped thanks to pairs of near-isogenic lines differing only in the region of the interesting gene (Philouze 1991; Laterrot 1996). Nevertheless, until the 1980s, the location of mutations of interest on genetic maps was not precise. The first isozyme markers were limited in number and rapidly replaced by restriction fragment length polymorphism (RFLP) markers. The first high-density genetic map based on RFLP markers was constructed (Tanksley et al. 1992). With more than 1000 loci, spread on the 12 chromosomes, it allowed the localization of several mutations and genes of interest. Then, PCR-based markers, including random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and microsatellites, were used, but remained limited in polymorphism level and distribution across the genome. Following the identification of PCR markers linked to the gene of interest, specific PCR markers were set up, simplifying the genotyping step for breeders. Nevertheless, PCR markers such as RAPD or AFLP map in majority close to the centromeres, reducing their potential efficiency for gene mapping in tomato (Grandillo and Tanksley 1996a; Haanstra et al. 1999; Saliba-Colombani et al. 2001).

2.3.2.2 Trait Mapping

The construction of genetic maps of molecular markers permitted the dissection of quantitative traits into QTLs (quantitative trait loci) (Paterson et al. 1988; Tanksley et al. 1992). This strategy also opened the way to investigate physical mapping and molecular cloning of genetic factors underlying quantitative traits (Paterson et al. 1991). The first gene cloned by positional cloning was the *Pto* gene, conferring resistance to *Pseudomonas syringae* (Martin et al. 1994). Since then, several interspecific progenies with each wild relative species were studied. Due to the low genetic diversity within the cultivated compartment (Miller and Tanksley 1990), most of the mapping populations were based on interspecific crosses between a cultivar and a related wild species from the lycopersicon group (as reviewed by Foolad 2007; Labate et al. 2007; Grandillo et al. 2011) or from lycopersicoides (Pertuzé et al. 2003) and juglandifolia group (Albrecht et al. 2010). However, maps based on intraspecific crosses have proved their interest notably for fruit quality aspects (Saliba-Colombani et al. 2001). All those populations allowed the discovery and characterization of a myriad of major genes (Rothan et al. 2019) and QTLs involved in various traits (Grandillo and Tanksley 1996b; Tanksley et al. 1996; Fulton et al. 1997; Bernacchi et al. 1998a, b; Chen et al. 1999; Grandillo et al. 1999; Frary et al. 2000; Monforte and Tanksley 2000; Causse et al. 2001; Saliba-Colombani et al. 2001; Causse et al. 2002; Doganlar et al. 2003; Frary et al. 2004; Schauer et al. 2006; Baldet et al. 2007;

Jiménez-Gómez et al. 2007; Cagas et al. 2008; Kazmi et al. 2012a, b; Haggard et al. 2013; Alseekh et al. 2015; Pascual et al. 2015; Ballester et al. 2016; Rambla et al. 2014; Kimbara et al. 2018).

The main results of QTL studies can be summarized:

- QTLs are detected in every case, sometimes with strong effects. A few QTLs explaining a large part of the phenotypic variation, acting together with minor QTLs, are frequently detected. Most of the QTLs act in an additive manner, but a few dominant and even overdominant QTLs were detected (Paterson et al. 1988; DeVicente and Tanksley 1993).
- QTLs can be separated into two types: QTLs stable over the environments, years or types of progeny, and QTLs more specific of one condition (Paterson et al. 1991).
- Some regions involved in the variation of a trait are found in progenies derived from different accessions of a species, or from different species (Fulton et al. 1997; Bernacchi et al. 1998a, b; Chen et al. 1999; Grandillo et al. 1999; Fulton, 2002).
- The dissection of complex traits in relevant components and the QTL mapping of these components allowed the genetic bases of the variability of complex traits to be understood. For example, a map of QTLs controlling several attributes of organoleptic quality in fresh-market tomato revealed relations between QTLs for sensory attributes and chemical components of the fruit (Causse et al. 2002). The analysis of biochemical composition of a trait is also important.
- Fine mapping experiments allowed to precisely map the QTLs in a chromosome region and to verify the existence of several QTLs linked in the same region (Paterson et al. 1990; Frary et al. 2003; Lecomte et al. 2004a). For example, by reducing the size of introgressed fragments from *S. pennellii*, (Eshed and Zamir 1995) identified three linked QTLs controlling fruit weight on a single chromosome arm. Fine mapping is also an important step for cloning QTLs, as first shown by the successes in cloning QTLs controlling fruit weight (Alpert and Tanksley 1996; Frary et al. 2000), fruit shape (Tanksley 2004) and soluble solid content (Fridman et al. 2000, 2004).
- Wild species, in spite of their low characteristics in comparison to cultivars, can carry alleles, which may contribute to the improvement of most of the agronomic traits (DeVicente and Tanksley 1993).

2.3.2.3 Specific Populations to Dissect Phenotypes

Rapidly, molecular breeding strategies were set up and implemented to try to "pyramid" genes and QTL of interest for agronomical traits, notably using advanced backcross QTL method (AB-QTL) (Grandillo and Tanksley 1996b). Using this approach with a *S. lycopersicum x S. pimpinellifolium* progeny, in which agronomical favorable QTL alleles were detected, Grandillo and colleague*s* showed how a wild species could contribute to improve cultivated tomato (Grandillo et al. 1996). Introgression

Lines (IL) derived from interspecific crosses allowed to dissect the effect of chromosome fragments from a donor (usually from a wild relative) introgressed into a recurrent elite line. IL offers the possibility to evaluate the agronomic performance of a specific set of QTL (Paran et al. 1995). IL was used as a base for fine mapping and positional cloning of several genes and QTL of interest. The first IL library was developed between *S. pennellii* and *S. lycopersicum* (Eshed and Zamir 1995; Zamir 2001). QTL mapping power was increased compared to biallelic QTL mapping population, and was again improved by the constitution of sub-IL set with smaller introgressed fragments. This progeny was successful in identifying QTLs for fruit traits (Causse et al. 2004); antioxidants (Rousseaux et al. 2005), vitamin C (Stevens et al. 2007), and volatile aromas (Tadmor et al. 2002). The introgression of a QTL identified in these IL has allowed plant breeders to boost the level of soluble solids (brix) in commercial varieties and largely increased tomato yield in California (Fridman et al. 2004). Complementary genetic resources are now available, including a new backcrossed inbred line (BIL) population generated by repeated backcrosses, followed by selfing (Ofner et al. 2016). This BIL population could be used in combination with ILs for fine mapping QTLs previously identified and to pinpoint strong candidate genes (Fulop et al. 2016). Moreover, the *S. pennellii* ILs have been broken into additional sublines carrying molecular marker-defined introgressions that are smaller than those carried by the original ILs, further facilitating the identification of candidate genes (Alseekh et al. 2013). These sub-isogenic lines are available to the scientific community and have been used to map loci affecting fruit chemical composition (Alseekh et al. 2015; Liu et al. 2016a, b). Such exotic libraries were also designed with other species, involving *S. pimpinellifolium* (Doganlar et al. 2003), *S. habrochaites* (Monforte and Tanksley 2000; Finkers et al. 2007a, b), and *S. lycopersicoides* (Canady et al. 2005).

Introgression lines were also used to dissect the genetic basis of heterosis (Eshed and Zamir 1995). Heterosis refers to a phenomenon where hybrids between distant varieties or crosses between related species exhibit greater biomass, speed of development, and fertility than both parents (Birchler et al. 2010). Heterosis involves genome-wide dominance complementation and inheritance model such as locus-specific overdominance (Lippman and Zamir 2007). Heterotic QTL for several traits were identified in tomato IL (Semel et al. 2006). A unique QTL was shown to display at the heterozygous level improved harvest index, earliness, and metabolite content (sugars and amino acids) in processing tomatoes (Gur et al. 2010, 2011). Furthermore, a natural mutation in the SFT gene, involved in flowering (Shalit et al. 2009), was shown to correspond to a single overdominant gene increasing yield in hybrids of processing tomato (Krieger et al. 2010).

2.3.2.4 Genes and QTLs Controlling Tomato Disease Resistance

The excessive use of chemical fungicides and pesticides was for a long time most common in tomato crops. Because of environmental, consumer, and grower constraints, their elevated costs, and their limited effectiveness, other levers, such as

genetic resistance and various cultural practices, have to be integrated for achieving sustainable agriculture (Lefebvre et al. 2018). However, the development of new cultivars with enhanced resistance or tolerance was often hindered by the lack of genetic diversity within the cultivated *S. lycopersicum* germplasm, because of its narrow genetic diversity due to its domestication history. Screening the tomato-related wild species germplasm collections enabled to discover many sources of disease resistance traits during the last 80 years (Rick and Chetelat 1995). About 40 major resistance traits were discovered in wild tomato species. Those genes confer resistance to diseases of different pest and pathogen classes. Of the 40 major resistance traits, about 20 have been introgressed into cultivated tomato (Ercolano et al. 2012). *S. peruvianum*, *S. habrochaites*, *S. pimpinellifolium,* and *S. chilense* have proved to be the richest sources of resistance genes (Laterrot 2000). The systematic screening of tomato germplasm for disease resistance will probably permit to discover further novel resistance sources and consequently novel resistance loci (major resistance genes and resistance QTLs).

Resistance Gene and QTL Discovery

More than 100 loci underlying the 30 major tomato resistance diseases have been genetically mapped (Foolad and Panthe2012 for review). Molecular markers associated with many resistance genes or QTLs have been reported. Up to now, 26 major resistance genes were isolated (*Asc-1*, *Bs-4*, *Cf-2*, *Cf-4*, *Cf-5*, *Cf-9*, *Hero*, *I* (=*I-1*), *I-2*, *I-3*, *I-7*, *Mi-1.2* (= *Mi* = *Meu*), *ol-2*, *Ph-3*, *pot-1*, *Prf*, *Pto*, *Tm-1*, *Tm-2*, *Tm-2*^{2} (= *Tm-2.2* = *Tm-2*^{a}), *Ty-1, Ty-2*, *Ty-3*, *ty-5*, *Ve-1 (=Ve)*, *Sw-5*) (Table 2.3). Resistance tomato locus has a well-defined nomenclature; written in italic, they are abbreviated by 1–3 letters (the first letter in uppercase for dominant resistance alleles and in lowercase for recessive dominant alleles) and separated of a number by a dash, the number indicating the order of discovery of the gene for the target disease. In a few cases, the last figure is followed by a dot and another number indicating different alleles; alleles could also be indicated by a number or a letter in superscript.

Most of reported major effect resistance genes are dominant, except *pot-1, ty-5,* and *ol-2* conferring resistance to potyviruses (PVY and TEV), *Tomato yellow leaf curl virus* (TYLCV), and to *Oidium neolycoersici*, respectively, that were both cloned (Bai et al. 2008; Lapidot et al. 2015; Ruffel et al. 2005). Another recessive resistance allele *py-1* (also named *pyl*) controlling *Pyrenochaeta lycopersici* responsible for corky root rot was reported but is not cloned yet (Doganlar et al. 1998).

For a few tomato diseases, both major effect resistance genes and resistance QTLs have been identified according to the resistance genitor and the pathogen variant used in the analysis and to environmental conditions. Otherwise, a single major resistance gene was discovered for most tomato diseases. For a few diseases, several major resistance genes have been reported, such as for TSWV, where 6 dominant resistance genes and 3 recessive resistance genes were described (Foolad and Panthee 2012) and for *Meloidogyne* nematodes where several resistance genes have been identified.

Table 2.3 Pest and pathogen resistance genes of tomato molecularly characterized. Genes are classified by pest and pathogen Latin name inside each pest and pathogen class. For each gene, the ITAG gene model(s) and the Genebank accession number are given when available

Locus name (synonym)	Function of cloned gene	Species from which the trait was discovered	Genetic resources carrying this gene	Tomato chromosome	ITAG gene model	Genebank accession number	Literature
Asc (Asc-1)	LAG1 Longevity Assurance Gene Family	*S. pennellii*	VFNT Cherry, LA716	T3	Solyc03g114600	AJ312131	Brandwagt et al. (2000)
Cf-2	Leucine-rich repeat receptor-like protein kinase LRR-RLP	*S. pimpinellifolium*	LA2244, LA3043	T6	Solyc06g008300	U42444	Dixon et al. (1996)
Cf-4	Leucine-rich repeat receptor-like protein kinase LRR-RLP	*S. habrochaites*	LA2446, LA3045, LA3051, LA3267	T1	Solyc01g006550	AJ002235	Takken et al. (1998, 1999)
Cf-5	Leucine-rich repeat receptor-like protein kinase LRR-RLP	*S. lycopersicum*	–	T6	–	AF053993	Dixon et al. (1998)
Cf-9	Leucine-rich repeat receptor-like protein kinase LRR-RLP	*S. pimpinellifolium*	LA3047	T1	Solyc01g005160	AJ002236	Jones et al. (1994)

(continued)

Table 2.3 (continued)

Locus name (synonym)	Function of cloned gene	Species from which the trait was discovered	Genetic resources carrying this gene	Tomato chromosome	ITAG gene model	Genebank accession number	Literature
I (I-1)	Leucine-rich repeat receptor-like protein kinase LRR-RLP	*S. pimpinellifolium*	PI79532	T11	Solyc11g011180		Catanzariti et al. (2017)
I-2	CC-NB-LRR	*S. pimpinellifolium*	PI126915	T11	Solyc11g071430		Ori et al. (1997), Simons et al. (1998)
I-3	S-receptor-like kinase 5 (SRLK-5)	*S. pennellii*	LA716	T7	Solyc07g055640	KP082943	Catanzariti et al. (2015)
I-7	Leucine-rich repeat receptor-like protein kinase LRR-RLP	*S. pennellii*	PI414773, Tristar cultivar	T8	Solyc08g77740	KT185194	Gonzalez-Cendales et al. (2016)
ol-2 (SlMlo1)	Loss-of-function mlo	*S. lycopersicum*	LA1230, KNU-12 cultivar	T4	Solyc04g049090	AY967408	Bai et al. (2008)
Ve-1 (Ve)	RLP-type resistance protein	*S. lycopersicum*	VFN8, Craigella GCR 151, PI 303801	T9	Solyc09g005090	AF272367	Kawchuk et al. (2001), Fradin et al. (2009)
Ph-3	CC-NB-LRR	*S. pimpinellifolium*	LA4285, LA4286, LA1269(= PI365957), L3708	T9	near Solyc09g092280-Solyc09g092310	KJ563933	Zhang et al. (2013, 2014)

(continued)

Table 2.3 (continued)

Locus name (synonym)	Function of cloned gene	Species from which the trait was discovered	Genetic resources carrying this gene	Tomato chromosome	ITAG gene model	Genebank accession number	Literature
pot-1	eukaryotic translation initiation factor 4E (eIF4E)	*S. habrochaites*	PI247087	T3	Solyc03g005870	AY723736	Ruffel et al (2005), Piron et al. (2010)
Tm-1	Inhibitor of tobamovirus RNA replication	*S. habrochaites*	PI126445	T2	Solyc02g062560	AB713135, AB713134	Ishibashi et al. (2007)
Tm-2	CC-NB-LRR	*S. peruvianum*	Craigella GCR236	T9	Solyc09g018220	AF536200	Lanfermeijer et al. (2005)
Tm-2^2 (Tm-2^a)	CC-NB-LRR	*S. peruvianum*	Craigella GCR267	T9	Solyc09g018220	AF536201	Lanfermeijer et al. (2005)
Sw-5	CC-NB-LRR	*S. peruvianum*	PI128654/Stevens cultivar	T9	Solyc09g098130	AY007367	Brommonschenkel et al. (2000)
Ty-1	DFDGD-Class RNA–Dependent RNA Polymerases	*S. chilense*	LA1969	T6	Solyc06g051170, Solyc06g051180, and Solyc06g051190		Verlaan et al. (2013)
Ty-2 (TYNBS1)	CC-NB-LRR	*S. habrochaites*	H9205, TY-Chie, Shurei cultivars	T11	near Solyc11g069660.1 and Solyc11g069670.1	LC126696	Yamaguchi et al. (2018)
Ty-3	DFDGD-Class RNA–Dependent RNA Polymerases	*S. chilense*	LA2279	T6	Solyc06g051170, Solyc06g051180, and Solyc06g051190		Verlaan et al. (2013)

(continued)

Table 2.3 (continued)

Locus name (synonym)	Function of cloned gene	Species from which the trait was discovered	Genetic resources carrying this gene	Tomato chromosome	ITAG gene model	Genebank accession number	Literature
ty-5	messenger RNA surveillance factor Pelota (Pelo)	*S. peruvianum*	Tyking cultivar TY172	T4	Solyc04g009810	KC447287	Lapidot et al. (2015)
Pto	Serine/threonine protein kinase	*S. pimpinellifolium*	LA2396, LA2458, LA3472	T5	Solyc05g013300	U02271	Martin et al. (1993)
Prf	CC-NB-LRR	*S. pimpinellifolium*	LA2396, LA2458, LA3472	T5	Solyc05g013280	U65391	Salmeron et al. (1996)
Bs-4	TIR-NB-LRR	*S. lycopersicum*	Money Maker cultivar	T5	Solyc05g007850	AY438027	Schornack et al. (2004)
Hero	CC-NB-LRR	*S. pimpinellifolium*	LA121	T4	Solyc04g008120	AJ457051	Ernst et al. (2002)
Mi-1.2 (Mi, Meu)	CC-NB-LRR	*S. peruvianum*	Motelle cultivar and most of tomato rootstocks	T6	Several homologs on Chr6	AF039682	Vos et al. (1998), Milligan et al. (1998), Nombela et al. (2001), Rossi et al. 1998, Casteel et al. (2007)

However, generally a single of those genes, such as *Sw-5* and *Mi-1.2*, is currently used in MAS because it confers a broader spectrum resistance than others.

A few cloned genes correspond to allelic series such as *Ty-1* and *Ty-3* on chromosome T6 (Verlaan et al. 2013), or *Tm-2* and *Tm-2*^{2} on chromosome T9 (Lanfermeijer et al. 2005), to very tightly linked genes such as *Pto* and *Prf* on chromosome T5 both involved in recognition of *Pseudomonas syringae* pv. *tomato* (Salmeron et al. 1996a, b), or else they belong to clusters of major resistance genes such as *Cf-4* and *Cf-9* on chromosome T1 (Takken et al. 1999) or *Cf-2* and *Cf-5* on chromosome T6 (Dixon et al. 1998). Additionally, while resistance genes are often specific to a pest, a pathogen, or a variant of a species, in rare cases, a same gene can confer resistance to different distantly related pests, such as *Mi-1.2* called also *Meu* that triggers the resistance to root knot nematodes caused by three *Meloigogyne* species (*M. incognita, M. arenaria, M. javanica*), to the aphid *Macrosiphum euphorbiae*, to the whitefly *Bemisia tabaci*, and to the psyllid *Bactericerca cockerelli* (Casteel et al. 2007; Milligan et al. 1998; Nombela et al. 2003; Rossi et al. 1998; Vos et al. 1998).

For many diseases, no major gene has been found yet, or major genes previously discovered were breakdown by virulent pathogen variants. For this reason, several research groups are now willing to focus on quantitative resistance that has the particularity to reduce the development of pests and pathogens rather than to block them totally. Quantitative resistance, also called partial resistance and generally controlled by QTLs, provides in most of the cases a more durable and broad-spectrum resistance (Cowger and Brown 2019); in addition, resistance QTLs are more frequent than major resistance genes in natural genetic resources. Many resistance QTLs have been mapped in the tomato genome, particularly for resistance traits to *P. infestans* (Arafa et al. 2017; Brouwer et al. 2004; Brouwer and St Clair 2004; Foolad et al. 2008; Ohlson et al. 2018; Ohlson and Foolad 2016; Panthee et al. 2017; Smart et al. 2007), *O. lycopersici* (Bai et al. 2003), *Alternaria solani* (Foolad et al. 2002), *Alternaria alternata* (Robert et al. 2001), *Xanthomonas sp.* (Hutton et al. 2010; Sim et al. 2015), *C. michiganensis* (Coaker and Francis 2004; Kabelka et al. 2002), *Ralstonia solanacearum* (Carmeille et al. 2006; Mangin et al. 1999; Wang et al. 2013a, b), *Botrytis cinerea* (Davis et al. 2009; Finkers et al. 2008; Finkers et al. 2007a, b) and *Cucumber mosaic virus* (CMV) (Stamova and Chetelat 2000).

Mainly, three genes were described for controlling resistance to late blight, but *Ph-1* is not effective anymore, due to the emergence of evolved races of *P. infestans*, and *Ph-2* and *Ph-3* have both an incomplete penetrance and evolved races of *P. infestans* have been described on plant material carrying those genes. Due to the breakdown of those three major resistance genes controlling late blight, many efforts are now underway to identify new resistance sources in tomato relatives and within the cultivated tomato germplasm (Caromel et al. 2015 and work in progress at INRA GAFL; Foolad et al. 2014).

An approach to breed for resistance when there are no natural variants, without transformation with foreign DNA, consists to inactivate by TILLING plant dominant susceptibility genes that permit the pathogen to multiply. A proof of concept of such an approach has allowed the de novo creation of resistance to two potyvirus species in tomato (Piron et al. 2010). Similarly, EcoTILLING allows the detection of natural

variability of the allelic variants of a specific gene, an approach that has resulted in the detection in tomato diversity of a new Sw-5 variant controlling TSWV (Belfanti et al. 2015).

Resistance Gene and QTL Architecture

Mapping of resistance loci in the tomato genome highlights several hotspots of resistance genes even if the 12 tomato chromosomes harbor resistance loci (Fig. 2.3). Equally, mapping of the repertoire of major resistance genes evidenced that they are organized in tandem or in clusters (Foolad 2007). It appears that a lot of resistance loci were identified on chromosomes 6 and 9, from the same genitor or from the tomato wild relatives. The chromosome 6 carries major resistance genes to root knot *Meloidogyne* (*Mi-1.2*), *O. neolycopersici* (*Ol-1*, *Ol-3*, *Ol-4*, *Ol-5* and *Ol-6*), *Cladosporium fulvum* (*Cf-2* and *Cf-5*), TYLCV (*Ty-1* and *Ty-3*), *Alfalfa mosaic virus* (*Am*), and resistance QTLs to *Ralstonia solanacearum* and ToMoV (*Tomato mottle virus*) (Agrama and Scott 2006). Identically the chromosome 9 is rich in resistance gene clusters with *Tm-2* and *Tm-2*^{2} controlling the *Tomato mosaic virus* (ToMV) (Pillen et al. 1996) and *Frl* controlling FORL (Vakalounakis et al. 1997) near the centromere, *Sw-5* controlling TSWV (Stevens et al. 1995) and *Ph-3* controlling *P. infestans* (Chunwongse et al. 2002) near a telomere, and *Ve* controlling *Verticillium dahliae* near the other telomere (Kawchuk et al. 2001).

Molecular Basis of Resistance Genes and QTLs

Many resistance traits in tomato are conferred by single dominant genes, encoding proteins that recognize directly or indirectly avirulent proteins of pests and pathogens and trigger the plant defense response. A few correspond to single recessive genes (e.g., *pot-1*, *ol-2*, generally written with lowercase letters). Recessive resistance alleles are due to loss-of-function or absence of susceptibility that hampers the pathogen's development in the plant; conversely, the corresponding susceptible alleles facilitate the development of the pathogen that benefits of the host's machinery. Many major resistance genes have been cloned by forward genetics and map-based cloning approaches (see Sect. 3.6 below) and most of the dominant cloned genes encode conserved NB-LRR proteins. The conserved molecular structure of resistance genes (NB-LRR R-genes, RLP, RLK, etc.) was used to search for genes homologous to genes already isolated in the same species or in related species, and to discover and isolate new resistance alleles or genes (e.g., *Sw-5* and *Mi* that are homolog, the *Cf* serie genes). More recently, the RenSeq technology, using baits designed from 260 NBS-LRR genes previously identified in Solanaceae, helped to pick-up 105 novel nucleoside binding site-Leicin rich repeat (NBS-LRR) sequences within the reference genome of tomato (*S. lycopersicum*) Heinz1706 and 355 novel NBS-LRR novel within the draft of *S. pimpinellifolium* LA1589 genome, to complete the repertoire of genes that encode NB-LRR R-genes in these species (Andolfo et al. 2014).

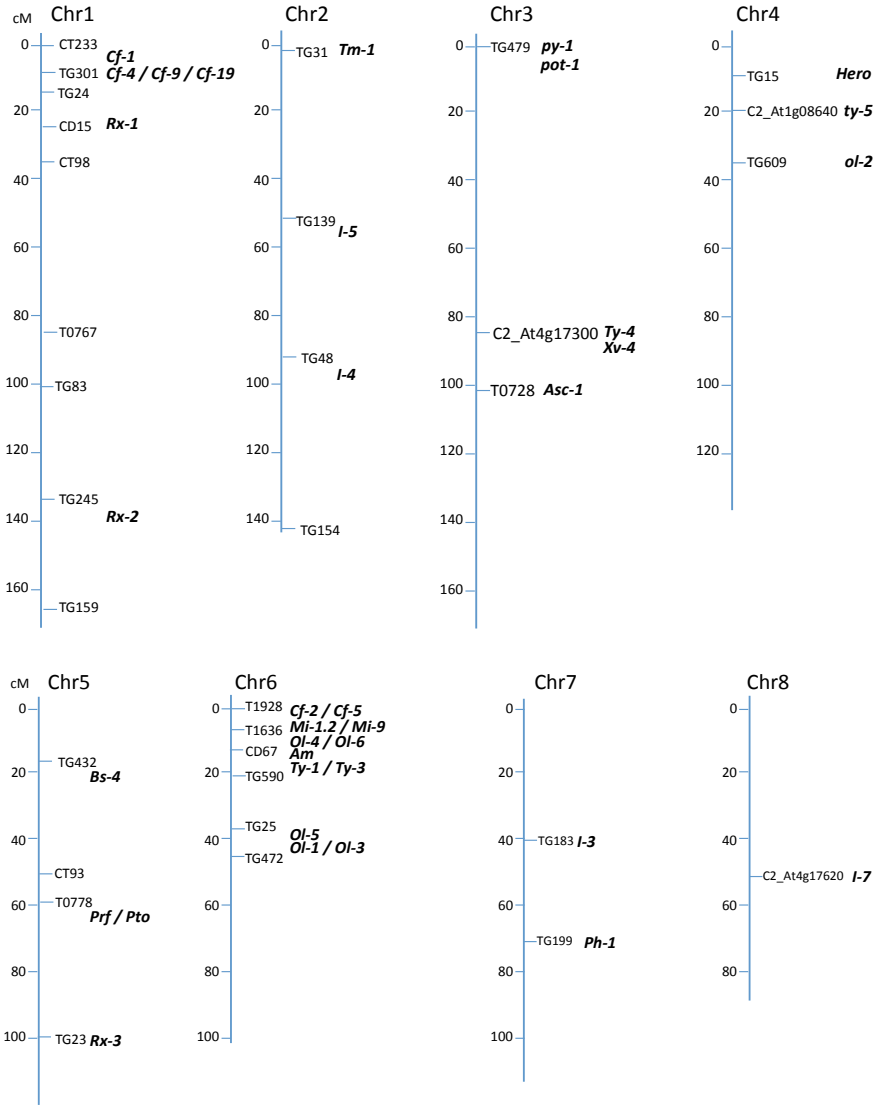

Fig. 2.3 Genetic map of tomato with mapped major resistance genes. Marker names and genetic distances are according to the SGN tomato- EXPEN 2000 map (https://solgenomics.net/). The position of genes is adapted from Foolad (2007), Foolad et al. (2014), Lee et al. (2015), Bai et al. (2018), Gill et al. (2019) and Sharma et al. (2019). When there is no common marker between the publication and the EXPEN 2000 map, the relative position was determined using a blastn search with the linked marker sequences as a query, against tomato chromosomes SL2.50 to identify the nearest marker. Genetic distances (in cM) are indicated on the left of the chromosomes

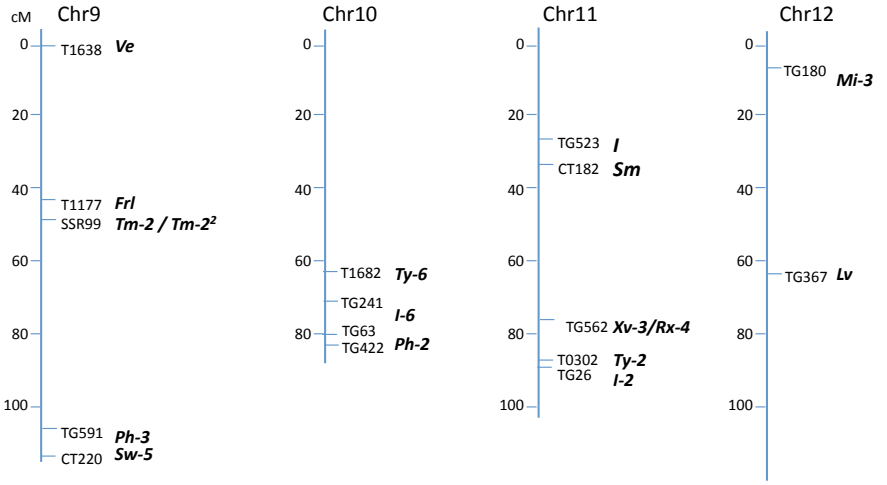

Fig. 2.3 (continued)

Besides those major effect resistance genes, many genes activated during the tomato disease defense response were also characterized. Several are specific of a plant–pathogen interaction. A few are involved in several plant–pathogen interactions, such as the lipase-like protein EDS1 that is involved in defense mechanisms triggered by Cf-4 and Ve proteins. Equally Prf, I-2, and Bs-3 proteins interact with the RAR1, SGT1, and HSP90 proteins. Beside, transcriptional analysis highlighted several genes involved in jasmonate acid or salicylic acid signaling pathway regulation. A few of these genes could correspond to resistance QTLs.

Until now, no QTL determining disease resistance has been cloned in tomato. Quantitative plant resistance loci may correspond to a large array of molecular mechanisms that play a role in partial resistance, they may be genes involved in PAMP recognition responsible for basal defense, genes involved in defense signal transduction, genes regulating the phytoalexin synthesis, weak effect alleles of R-genes, genes regulating developmental phenotypes, or other genes not yet identified (Poland et al. 2009).

2.3.3 Genomic Resources

2.3.3.1 The Reference Genome Sequence

Genomic information greatly promoted our understanding of the genetic architecture and evolutionary history of modern tomato. The tomato genome sequencing project was initiated as part of the International Solanaceae Project (SOL), which was launched on November 3, 2003 at Washington, USA and gathered a consortium

of scientists of 10 countries including China, France, Spain, Italy, USA, UK, the Netherlands, Japan, Korea, and India (Mueller et al. 2005). The main reason why tomato was first chosen as the reference genome for the Solanaceae was due to its high level of macro and micro-synteny among over 3,000 species. This project was first started with conventional sequencing technologies, such as Sanger sequencing. In order to reduce the cost of producing a high-quality reference, bacterial artificial chromosome (BAC)-by-BAC sequencing strategy based on saturated genetic markers was used to select seed BACs within the gene-rich part of the tomato genome for sequencing. However, this process was quite slow and became a serious obstacle, which was greatly accelerated by next-generation sequencing.

The first tomato genome sequence was published in 2012 for the inbred tomato cultivar "Heinz 1706" (*S. lycopersicum*) together with a draft of its closest wild species *S. pimpinellifolium* (accession LA1589) (The Tomato Genome Consortium 2012). In the tomato genome, recombination, genes, and transcripts are substantially located in the euchromatin regions compared to the heterochromatin regions, whereas chloroplast insertions and conserved microRNA genes were more evenly distributed throughout the genome (The Tomato Genome Consortium 2012). The tomato genome was highly syntenic with other Solanaceae species, such as pepper, eggplant, potato, and *Nicotiana*. Tomato had fewer high-copy, full-length long terminal repeat retrotransposons with older insertion ages compared to *Arabidopsis* and Sorghum. Genome annotation showed that there were a total 34,727 protein-coding genes and 30,855 of them were supported by RNA sequencing data. Chromosomal organization of genes, transcripts, repeats, and sRNAs were very similar between tomato and potato. Among all the protein-coding genes, 8,615 genes were common to tomato, potato, *Arabidopsis*, rice, and grape. A total of 96 conserved sRNAs were predicted in tomato, which could be further divided into 34 families, 10 of which being highly conserved in plants. The potato genome showed more than 8% divergence from tomato, with nine large and several smaller inversions (The Tomato Genome Consortium 2012). The *Solanum* lineage has experienced one ancient and one more recent consecutive genome triplication. The genome information provides a basic understanding of the genetic bottlenecks that narrowed tomato genetic diversity (The Tomato Genome Consortium 2012).

Since the first published version, the sequence has been completed, corrected, and re-annotated using new sequence data and new RNAseq data and the genome version today is SL3.0 while the annotation is ITAG3.2.

2.3.3.2 Resequencing Tomato Accessions

Next-generation sequencing technologies made it possible to sequence genomes at large scales (Goodwin et al. 2016). Soon after the availability of the reference tomato genome, the genome of the stress-tolerant wild tomato species *S. pennellii* was published (Bolger et al. 2014). This species is characterized by extreme drought tolerance and unusual morphology. Many stress-related candidate genes were mapped in this wild species. Large gene expression differences were observed between *S.*

lycopersicum cv. M82 and *S. pennellii* (LA716) due to polymorphisms at the promoter and/or coding sequence levels. This wild species and others were further re-sequenced and assembled using long read sequencing platforms complemented with Illumina sequencing (Usadel et al. 2017). After the genome of *S. pennellii*, a panel of diversified tomato accessions and related wild species were sequenced (The 100 Tomato Genome Sequencing Consortium 2014). The allogamous self-incompatible wild species have the highest level of heterozygosity, which was low for the autogamous self-compatible species (The 100 Tomato Genome Sequencing Consortium 2014). Almost at the same time, a comprehensive genomic analysis based on resequencing 360 tomato accessions elucidated the history of tomato breeding (Lin et al. 2014). This study showed that domestication and improvement of tomato mainly involved two independent sets of QTLs leading to fruit size increase. Five major QTLs (*fw1.1*, *fw5.2*, *fw7.2*, *fw12.1,* and *lcn12.1*) contributed to the enlargement of tomato fruit during the domestication process. Then, up to 13 major QTLs (*fw1.1*, *fw2.1*, *fw2.2*, *fw2.3*, *lcn2.1*, *lcn2.2*, *fw3.2*, *fw3.2*, *fw5.2*, *fw7.2*, *fw9.1*, *fw10.1*, *fw11.1*, *fw12.1*, *fw11.3*, *fw12.1,* and *lcn12.1*) contributed to the second improvement of tomato fruit. This study also detected several independent mutations in a major gene *SlMYB12* that changed modern red tomato to pink tomato appreciated in Asia. This study also illustrated the linkage drag associated with wild introgressions (Lin et al. 2014).

Since then, low-depth resequencing or genotyping-by-sequencing has become a common practice and is widely applied in many tomato collections. Up to now, around 900 tomato accessions have been re-sequenced, with the sequence depth ranging from low to high (The Tomato Genome Consortium 2012; Causse et al. 2013; Bolger et al. 2014; Lin et al. 2014; The 100 Tomato Genome Sequencing Consortium 2014; Tieman et al. 2017; Ye et al. 2017; Tranchida-Lombardo et al. 2018). These genomic resources are freely available (https://solgenomics.net) and will greatly facilitate modern breeding of new climate-smart tomato cultivars.

In a recent pan-genome study of 725 phylogenetically and geographically representative tomato accessions, a total of 4,873 genes were newly discovered compared to the reference genome (Gao et al. 2019). Among these, 272 were potential contaminations and were removed from the "Heinz 1706" reference genome. Substantial gene loss and intensive negative selection of genes and promoters were detected during tomato domestication and improvement. During tomato domestication, a total of 120 favorable and 1,213 unfavorable genes were identified, whereas 12 favorable and 665 unfavorable genes were identified during the improvement process.

Disease resistance genes were especially lost or negatively selected. Gene enrichment indicated that defense response was the most enriched group of unfavorable genes during both domestication and improvement. No significantly enriched gene families were found in favorable genes during improvement. A rare allele in the *TomLoxC* promoter was found under selected during domestication. In orange-stage fruit, accessions with both the rare and common *TomLoxC* alleles have high expression compared to those homozygous in modern tomatoes. Taken together with other

findings, this pan-genome study provides useful knowledge for further biological discovery and breeding (Gao et al. 2019).

2.3.4 SNP Markers

2.3.4.1 SNP Discovery

Single nucleotide polymorphisms (SNPs) are the most abundant molecular markers for major crops. SNPs can be detected in any region of the genome, including coding sequences or non-coding sequences of genes, as well as the intergenic regions. Only the non synonymous SNPs in the coding regions of genes change the amino acid sequences of proteins. However, SNPs in the non-coding region are also likely to affect gene expression through different mechanisms (Farashi et al. 2019). Millions of SNPs can be directly generated via genotyping-by-sequencing (GBS) or resequencing of a few lines (Catchen et al. 2011). Next-generation sequencing-based technologies have also accelerated the identification and isolation of genes associated with agronomic traits in major crops (Le Nguyen et al. 2018). There are many GBS methods available, including at least 13 reduced-representation sequencing (RRS) approaches and at least four whole-genome resequencing (WGR) approaches (Scheben et al. 2017). Among them, RNA sequencing and exome sequencing based on transcriptome sequences is an important alternative RRS approach (Haseneyer et al. 2011; Scheben et al. 2017). The sequenced data can be used for expression analysis and also does not require prior genomic sequence information (Wang et al. 2010).

Since the availability of the reference tomato genome, whole-genome resequencing of different tomato accessions could directly generate millions of SNPs, covering the whole tomato genome (Bolger et al. 2014; Lin et al. 2014; Menda et al. 2014; The 100 Tomato Genome Sequencing Consortium 2014; Tieman et al. 2017; Ye et al. 2017; Zhu et al. 2018). The number of SNPs in the wild tomato species exceeds 10 million, which are 20-folds higher than that in most of the domesticated accessions (The 100 Tomato Genome Sequencing Consortium 2014). Once the reference genome was available, it became possible to only sequence chromosome regions of interest to screen for SNP. For example, Ranc et al. (2012) sequenced 81 DNA fragments covering the chromosome 2 at different mapping densities in a core collection of 90 tomato accessions and discovered 352 SNPs.

2.3.4.2 SNP Arrays

SNP arrays is another popular and cost-effective genotyping approach, such as the Solanaceae Coordinated Agricultural Project (SolCAP) (Hamilton et al. 2012; Sim et al. 2012b), the Centre of Biosystems Genomics (CBSG) consortium (Víquez-Zamora et al. 2013) or, the Diversity Arrays Technology (DArTseq) (Pailles et al.

2017). However, RNAseq based SNP arrays, such as SolCAP and ddRAD-Seq (Arafa et al. 2017), have some major limitations: Gene expression is dependent on tissue and time, multiple biases are introduced by library preparation during RNA fragmentation (Wang et al. 2009) and SNP coverage is low in coding regions (Scheben et al. 2017). In tomato, these SNP arrays have been widely used to genotype different tomato collections (Sim et al. 2012a; Víquez-Zamora et al. 2013; Ruggieri et al. 2014; Sauvage et al. 2014; Blanca et al. 2015; Bauchet et al. 2017a, b; Pailles et al. 2017; Albert et al. 2016b).

2.3.4.3 Genotype Imputation

When a large diverse reference panel is available, SNP density can be significantly increased by genotype imputation (Guan and Stephens 2008; Halperin and Stephan 2009; Iwata and Jannink 2010; Marchini and Howie 2010; Pasaniuc et al. 2012; Browning and Browning 2016; Das et al. 2016; Wang et al. 2018). In human and model plant species, there are some very good reference panels suitable for genotype imputation, such as the 1000 Genomes Project (The 1000 Genomes Project Consortium 2015) and the UK10K Project in humans (Danecek et al. 2015; The UK10K Consortium 2015), the 3000 Rice Genome Project (2014; McCouch et al. 2016), and the 1001 Genomes Consortium in *Arabidopsis thaliana* (2016). The marker density of SNP arrays in tomato is quite low and many genomic gaps remain, compared with the whole-genome sequencing (Sauvage et al. 2014; Bauchet et al. 2017b; Zhao et al. 2019). After imputation, the SNP number can be increased up to 30-folds and greatly bridged the genomic gaps and genomic coverage (Fig. 2.4) (Zhao et al. 2019).

2.3.5 Diversity Analyses

Molecular genetic markers play an important role in the modern breeding (Ramstein et al. 2018). They also provide a new vision of tomato genetic diversity (Bauchet and Causse, 2012). Overall, modern cultivated tomato accessions present a lower polymorphism level compared to wild species, as shown by different types of markers, such as RFLP (Miller and Tanksley, 1990), AFLP (Suliman-Pollatschek et al. 2002; Park et al. 2004; Van Berloo et al. 2008; Zuriaga et al. 2009), RAPD (Grandillo and Tanksley 1996a; Archak et al. 2002; Tam et al. 2005; Carelli et al. 2006; El-hady et al. 2010; Meng et al. 2010; Length 2011), SSR (Suliman-Pollatschek et al. 2002; Jatoi et al. 2008; Mazzucato et al. 2008; Albrecht et al. 2010; Meng et al. 2010; Sim et al. 2010; Zhou et al. 2015), ISSR (Vargas-Ponce et al. 2011; Shahlaei et al. 2014) and SNPs (Blanca et al. 2012; Sim et al. 2012a; Lin et al. 2014; The 100 Tomato Genome Sequencing Consortium 2014).

Whole-genome sequencing technology made it possible to detect millions of SNPs and it has revealed that the number of SNPs in wild species is over 10 million and is 20-fold higher than that for most domesticated tomato accessions (The

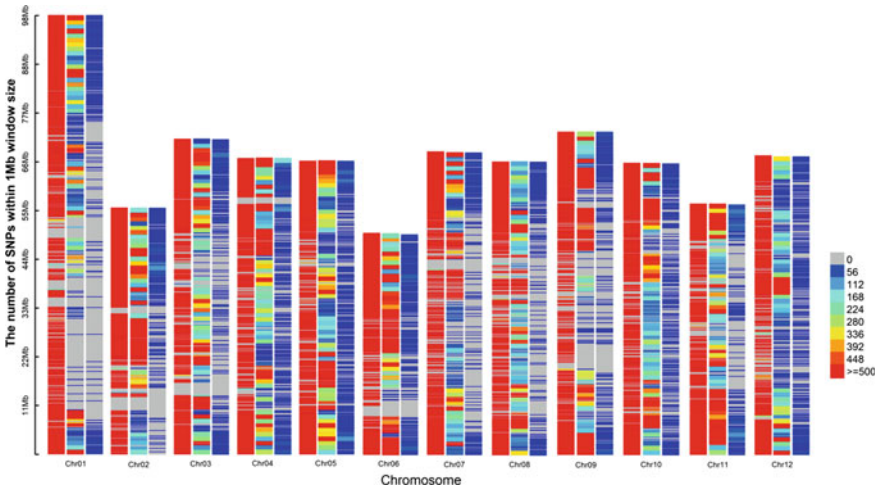

Fig. 2.4 SNP density for the tomato collection reported in Sauvage et al. (2014). Left, middle, and right panels represent the SNP density of the reference panel, after and before genotype imputation, adapted from Zhao et al. (2019)

100 Tomato Genome Sequencing Consortium 2014), which provides clues on the genetic diversity loss during tomato domestication and improvement. A study based on whole-genome sequencing of wild and cultivated tomato species demonstrated that approximately 1% of the tomato genome has experienced a very strong purifying selection during domestication (Sahu and Chattopadhyay 2017). At the expression level, domestication has affected up to 1729 differentially expressed genes between modern tomato varieties and the *S. pimpinellifolium* wild species and also affected about 17 gene clusters. Some gene regulation pathways were significantly enriched, such as carbohydrate metabolism and epigenetic regulations (Sauvage et al. 2017).

Cherry tomato accessions (*S. lycopersicum* var. *cerasiforme*) are intermediate between cultivated and wild species with a moderate genetic diversity (Ranc et al. 2012; Xu et al. 2013; Zhang et al. 2017). The linkage disequilibrium of cherry tomatoes is also intermediate between that of cultivated and wild species (Sauvage et al. 2014; Bauchet et al. 2017a). They could thus be helpful to bridge the gaps between low genetic diversity and high morphological diversity of modern cultivated tomato accessions and wild species which may provide interesting genes but also a strong genetic load. Molecular markers could also link the genetic and morphological diversities together and provide insight into the origin of tomato. By phenotyping 272 genetically and morphologically diverse tomato accessions with the SOLCAP genotyping SNP array, Blanca et al. (2012) revealed that cherry tomato accessions were morphologically and genetically intermediate between modern cultivated tomato accessions (*S. lycopersicum*) and wild accessions (*S. pimpinellifolium*). In addition,

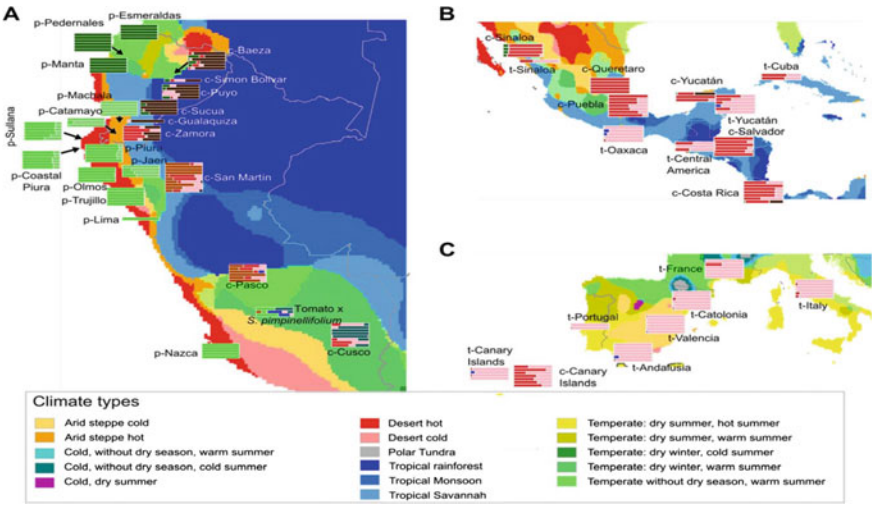

Fig. 2.5 Geographical distributions of the population structure revealed by SOLCAP SNPs, adapted from Blanca et al. (2012). Different colored bars represent the proportion of the population structure

cherry and wild tomato accessions inhabited strikingly different ecological and climatic regions and a clear relationship was found between the population structure and a geographic map based on the climatic classification (Fig. 2.5).

2.3.6 Cloned Genes/QTLs

Tomato is probably one of the crops with the largest number of single mutations used for its breeding (as reviewed by Grandillo and Cammareri (2016), and Rothan et al. 2019). Before the SNP discovery, due to the limited genetic diversity of domesticated tomato accessions, the populations used for linkage mapping have been generated by crosses between a cultivated and a close wild tomato species (Foolad 2007; Foolad and Panthee 2012). Since the development of molecular markers, these segregating populations have become an effective and efficient tool to construct high-density genetic linkage maps (Tanksley et al. 1992), allowing the detection of quantitative trait loci (QTLs). By using different linkage populations and multiple molecular markers, including RFLP, simple sequence repeat, (SSR) and SNPs, hundreds of QTLs have been reported, for different agronomical, morphological, and quality-related traits (Grandillo and Tanksley 1996b; Tanksley et al. 1996; Fulton et al. 1997; Bernacchi et al. 1998a, b; Chen et al. 1999; Grandillo et al. 1999; Fulton et al. 2000; Monforte and Tanksley 2000; Saliba-Colombani et al. 2001; Causse et al. 2002; Doganlar et al. 2003; van der Knaap and Tanksley 2003; Fridman et al. 2004; Baldet et al. 2007; Foolad 2007; Jiménez-Gómez et al. 2007; Cagas et al. 2008; Dal

Cin et al. 2009; Sim et al. 2010; Ashrafi et al. 2012; Haggard et al. 2013; Kinkade and Foolad 2013).

However, among the detected QTLs, only a few have been cloned and functionally validated (Bauchet and Causse 2012; Rothan et al. 2019). The first gene cloned by positional cloning in tomato was the *Pto* gene, conferring resistance to *Pseudomonas syringae* races, with the assistance of RFLP markers (Martin et al. 1994). Based on the same RFLP map, *Fen*, another member of this gene family, was also soon reported (Martin et al. 1994). From then on, different resistance genes were identified and cloned based on RFLP markers, such as *Cf-2*, a leucine-rich repeat protein conferring resistance to *Cladosopum fulvum* strains (Dixon et al. 1996); *Prf*, another resistance gene to *Pseudomonas syringae* pv. tomato (Pst) strains (Salmeron et al. 1996); *Ve* conferring Verticilium wilt resistance, encoding surface-like receptors (Kawchuk et al. 2001); and others. Some other markers were also developed and applied for resistance gene identification, such as *Ph-3* gene from *S. pimpinellifolium* conferring resistance to *Phytophthora infestans*, which was cloned based on cleaved amplified polymorphic sequences (CAPS) or insert/deletion (InDel) markers (Zhang et al. 2014). Sequence-characterized amplified region (SCAR) markers and cleaved amplified polymorphic sequence (CAPS) markers are also applying to map tomato yellow leaf curl virus resistance gene *Ty-2* (Yang et al. 2014).

Some important genes/QTLs involved in developmental processes were also identified and cloned with the assistance of molecular markers. Among them, *fw2.2*, a major QTL controlling tomato fruit weight, was one of the first examples. With the benefits of CAPs markers, a single candidate gene ORFX on chromosome 2 was identified and cloned (Frary et al. 2000), which alters tomato fruit size likely by expression regulation rather than sequence and structure variation of the encoded protein (Nesbitt and Tanksley 2002). Recently, some other major QTLs were functionally validated, such as *fw3.2* (corresponding to a cytochrome P450 gene) (Chakrabarti et al. 2013) and *fw11.2* (corresponding to a cell size regulator) (Mu et al. 2017). Some major QTLs closely related to fruit weight were also reported, such as *OVATE*, a negative regulatory gene causing pear-shaped tomato fruits (Liu et al. 2002); SUN, a retrotransposon-mediated gene (Xiao et al. 2008); locule number *fas* (Huang and van der Knaap 2011) and *lc* (Muños et al. 2011). Other cloned genes related to tomato development are summarized in a recent review paper (Rothan et al. 2019).

Tomato fruits are rich in diverse nutrients and health-promoting compounds, such as sugars, organic acids, amino acids, and volatiles (Goff and Klee 2006; Klee 2013). However, breed tomatoes with high nutrition and strong flavor still remain a major breeding challenge (Tieman et al. 2012; Klee and Tieman 2013; Klee and Tieman 2018; Zhao et al. 2019). *Lin5*, a major QTL modifying sugar content in tomato fruit, was cloned about 20 yearS ago (Fridman et al. 2000). In various genetic backgrounds and environments, the wild-species allele increased glucose and fructose contents compared to cultivated allele (Fridman et al. 2000). In addition, this gene shared a similar expression pattern in tomato, potato, and Arabidopsis (Fridman and Zamir 2003). Recently, a *SWEET* protein, a plasma membrane-localized glucose efflux transporter, was shown to play a role in the ratio of glucose and fructose accumulation (Shammai et al. 2018). A balanced content of sugars and organic acids is crucial for

consumer preference (Tieman et al. 2017). Recently, a major QTL regulating malate content was cloned, corresponding to an *Aluminium Activated Malate Transporter 9* (*Sl-ALMT9*) (Ye et al. 2017). In a new recent study, it was further found that this QTL was also likely regulating the content of citrate in tomato fruits (Zhao et al. 2019). Though only a few QTLs regulating sugars and organic acids have been functionally validated, this knowledge is important for understanding the regulation mechanisms. Several genes involved in the variation of volatile production were also characterized (Tieman et al. 2006; Tikunov et al. 2013; Klee 2010; Klee and Tieman 2018).

2.3.7 New Resources for Gene/QTL Identification

Lin et al. (2014) demonstrated the benefits of whole-genome resequencing of the two extreme bulk populations from an F_2 population of tomato, where many fruit weight QTLs were identified, including *fw2.1*, *fw2.2*, *fw2.3*, *lcn2.1*, *lcn2.2*, *fw9.1*, *fw9.3*, *fw11.1*, *fw11.2,* and *fw11.3*. Whole-genome sequencing of bulked F_2 plants with contrasted phenotypes offers the opportunity to identify the SNPs that are putatively related to the target phenotypes via aligning the sequenced data to the reference genome (Garcia et al. 2016). This approach has been efficient in identifying mutations, especially generated by EMS (Garcia et al. 2016).

However, the genetic diversity of linkage populations is limited to the two parental accessions used for crossing. In order to overcome this limitation, multi-parent advanced generation intercross (MAGIC) populations offer an alternative, which has been generated for different species, such as Arabidopsis (Kover et al. 2009), rice (Bandillo et al. 2013), wheat (Huang et al. 2012; Mackay et al. 2014), faba bean (Sallam and Martsch 2015), sorghum (Ongom and Ejeta 2017), and tomato (Pascual et al. 2015). The first tomato MAGIC population was developed by crossing eight re-sequenced tomato lines and there was no obvious population structure in this population. The linkage map was 87% larger than those derived from bi-parental populations and some major fruit quality QTLs were identified by using this approach (Pascual et al. 2015). Recently, this MAGIC population was also used for identifying QTLs under water deficit and salinity stresses and many stress-specific QTLs were identified (Diouf et al. 2018).

2.3.8 Genome-Wide Association Studies

2.3.8.1 The Conditions for Applying Genome-Wide Association Studies

Association mapping is used to detect associations between a given phenotype and genetic markers in a population of unrelated accessions. If the genetic markers cover the whole genome, it is referred to as genome-wide association studies (GWAS). This technology was first developed in humans. After the demonstration of GWAS

power to analyze human diseases (Klein et al. 2005), it was quickly adopted in major crops (Brachi et al. 2011; Luo 2015; Liu and Yan 2019). In tomato, the first reported association study was performed to identify the SNPs associated with the fruit weight QTL *fw2.2*. However, the authors did not find any positive associated SNP in a small collection of 39 cherry tomato accessions (Nesbitt and Tanksley 2002).

In order to efficiently apply GWAS in tomato, linkage disequilibrium (LD) in different tomato types was assessed using different molecular markers. In general, the LD in cultivated tomato accessions was larger than that of wild species, which could be up to about 20 Mbs, while cherry tomatoes ranged in between (Van Berloo et al. 2008; Mazzucato et al. 2008; Sim et al. 2010; Ranc et al. 2012; Xu et al. 2013; Sauvage et al. 2014; Zhang et al. 2016a, b; Bauchet et al. 2017a). These results also indicated that modern tomatoes lost genetic diversity during tomato domestication and breeding. Admixture of cherry tomatoes with modern cultivars and wild species could help reduce the large LD and overcome the low resolution of association mapping of modern tomato cultivars (Ranc et al. 2012). The average high degree of LD is beneficial in terms of the minimum number of molecular markers needed to cover the whole genome. For example, (Xu et al. 2013) performed an association mapping on 188 tomato accessions with 121 polymorphic SNPs and 22 SSRs. They successfully identified 132 significant associations for six quality traits. Before the availability of large SNP number, molecular markers such as SSRs were popular for GWAS. In particular, (Zhang et al. 2016a, b) genotyped 174 tomato accessions including 123 cherry tomato and 51 heirlooms with 182 SSRs and performed GWAS for fruit quality traits. A total of 111 significant associations were identified for 10 traits and many previously identified major QTLs were located in/near regions of the significant associated markers. The authors further extended the phenotypes to volatiles (Zhang et al. 2016a, b), as well as sugars and organic acids (Zhao et al. 2016). Many significant associations were also identified and some of them were consistent with other GWAS focusing on the same traits that were based on genome-wide SNPs (Sauvage et al. 2014; Bauchet et al. 2017b; Tieman et al. 2017; Zhao et al. 2019).

With the availability of the reference tomato genome (The Tomato Genome Consortium 2012), millions of SNPs became available and allowed the identification of causative polymorphisms. For instance, the causative gene *SlMYB12* conferring pink tomato fruit color was identified in a GWAS using 231 sequenced tomato accessions (Lin et al. 2014). Several mutations were further identified in the protein structure of SlMYB12 and the authors identified three recessive alleles of this gene useful for pink tomato breeding (Lin et al. 2014).

However, whole-genome-sequencing is still quite expensive, especially at a large population scale, which greatly limits the wide applications. SNP arrays were thus developed to overcome this limit (Hamilton et al. 2012; Sim et al. 2012b). Sauvage et al. (2014) genotyped 163 tomato accessions composed of large fruit, cherry, and wild tomato accessions with the SolCAP array, generating a total of 5995 high-quality SNPs. Then they performed GWAS using a multi-locus mixed model (MLMM; (Segura et al. 2012) for 36 metabolites that were highly correlated during two growth periods and identified 44 candidate loci associated with different fruit metabolites

(Sauvage et al. 2014). Among the candidate loci, they identified a gene with unknown function on chromosome 6 that was strongly associated with malate content. This association was further identified in different GWAS and meta-analysis of GWAS based on different populations (Bauchet et al. 2017b; Tieman et al. 2017; Ye et al. 2017; Zhao et al. 2019) and was further validated as an *Al-Activated Malate Transporter 9* (*Sl-ALMT9*) (Ye et al. 2017). In a meta-analysis of GWAS based on three populations, it was further found that this gene was also significantly associated with citrate content in tomato fruits, demonstrating its important role in the regulation of organic acids in tomato (Zhao et al. 2019). In fact, the Al-activated malate transporters are a family of plant-specific proteins, which are important for plant root tissue and function (Delhaize et al. 2007).

Bauchet et al. (2017b) genotyped 300 tomato accessions with both the SolCAP and CBSG arrays, generating a total of 11,012 high-quality SNPs, which were used for GWAS using both MLMM and multi-trait mixed model (MTMM) (Korte et al. 2012). A total of 79 significant associations were identified for 13 primary and 19 secondary metabolites in tomato fruits. Among these, two associations involving fruit acidity and phenylpropanoid content were particularly investigated (Bauchet et al. 2017b). The same population was also characterized for agronomic traits and many QTLs were identified, such as *fw2.2* and *fw3.2*. Within this panel, the authors also demonstrated that intermediate accessions shared different haplotype patterns compared to domesticated and wild tomatoes (Bauchet et al. 2017a). GWAS for similar quality traits were also performed in other collections (Ruggieri et al. 2014; Zhang et al. 2016a, b).

With the fast development of whole-genome-sequencing technology and the reduction of cost per genome, it is possible to sequence hundreds of diverse tomato collections. For instance, (Tieman et al. 2017) sequenced 231 new accessions and combined these data with 245 previously sequenced genomes, generating a total of 476 genome sequences. These data were then used for GWAS for diverse flavor-related metabolites, including 27 volatiles, total soluble solids, glucose, fructose, citric acid, and malic acid. A total of 251 significant associations were detected for 20 traits. Two loci were significantly associated with both glucose and fructose, corresponding to two major QTL *Lin5* and *SSC11.1*. By combining with selection analysis, it was further shown that the negative correlation between sugar content and fruit weight was likely caused by the loss of high-sugar alleles during domestication and improvement of ever-larger tomato fruits (Tieman et al. 2017). In addition, some good candidate genes involved in tomato volatile contents were also identified, such as Solyc09g089580 for guaiacol and methylsalicylate. By combining the three significant associated loci for geranylacetone and 6-methyl-5-hepten-2-one, it was shown that the allelic combinations conferring favorable aromas were progressively lost during domestication and breeding (Tieman et al. 2017).

2.3.8.2 Meta-Analysis

However, with the results of several GWAS in tomato for the same trait, only some significant associations could be identified in different studies, indicating strong cross-study heterogeneity, which refers to the non-random variance in the genetic effects between different GWASs. The main sources of heterogeneity include population structure, linkage disequilibrium, phenotyping measurement methods, environmental factors, genotyping methods, G×E interactions (Evangelou and Ioannidis, 2013). Meta-analysis of GWAS is a new approach to combine different GWAS properly handling the heterogeneity.

Zhao et al. (2019) reported the meta-analysis of GWAS from three tomato populations (Sauvage et al. 2014; Bauchet et al. 2017b; Tieman et al. 2017). Following genotype imputation, a total of 775 tomato accessions and 2,316,117 SNPs were used in the meta-analysis and a total of 305 significant associations were identified for the contents of sugars, organic acids, amino acids, and flavor-related volatiles. By looking at the five loci associated with both fructose and glucose, they showed that sugar contents significantly increased with the number of wild alleles. The authors also demonstrated that domestication and improvement have had an impact on citrate and malate content. In particular, the major QTL *Al-Activated Malate Transporter 9* of malate was also significantly associated with citrate and another malate transporter was identified for citrate content on chromosome 1. This study also identified many new significant associations for flavor-related volatiles. By targeting six significant associations, it was further demonstrated that modern tomato accessions had a limited flavor due to a lower content of pleasant volatiles but also a higher content of unpleasant volatiles compared to cherry tomatoes (Zhao et al. 2019).

2.3.9 Genetic Dissection of Abiotic Stress Tolerance

2.3.9.1 Genetic Control of GxE Interaction

In Sect. 2.3.2 above, the impact of different abiotic stresses on tomato was described. Nevertheless, a large diversity of response has been shown notably between the wild species and the cultivated one, but also across cultivated accessions. Several studies were conducted to understand the genetic mechanisms leading to such variation in tomato response to environmental stresses. Elucidating the genetic determinants of tomato response to abiotic stress was possible thanks to the high genetic diversity present in the *S. lycopersicum* clade.

A large panel of genetic resources is available for the tomato community, including both cultivated and wild species (Sect. 3.1). Screening the genetic diversity in both compartments brought to light high loss of diversity within the cultivated group (Lin et al. 2014) due to extensive directional selection toward agronomic performance traits. However, substantial diversity for environmental response genes remains in the cultivated group that could be attributed to local adaptations during the diversification

for both climatic conditions and growth conditions. This is identified by the presence of substantial genotype-by-environment (GxE) interactions, as observed in different intraspecific experimental tomato populations (Villalta et al. 2007; Mazzucato et al. 2008; Albert et al. 2016a; Diouf et al. 2018).

Besides, wild species constitute a reservoir of specific genes related to abiotic stress tolerance, derived from adaptation to their growing and typically harmful local habitats. For example, the two wild relative species *S. habrochaites* and *S. pennellii* are more tolerant to chilling stress (Bloom et al. 2004) and to drought and salinity stress conditions (Bolger et al. 2014), compared to cultivated species. The presence of tolerance genes in the wild species and the genetic diversity of stress-response genes in cultivated clade give clues to achieve considerable progress in tomato breeding for climate-smart cultivars.

Several studies investigated the genetic nature of tomato response to abiotic stresses since a high-density genetic map was made available. Grandillo et al. (2013) and Grandillo and Cammareri (2016) reported a summary of the QTLs that were identified under different abiotic stress conditions. Table 2.4 summarizes abiotic stress QTLs identified during the last decade only. These QTLs were mapped in different population types and with different mapping methods covering the wide range of mapping strategies available in plant genetics. These studies highlighted several phenotypic traits that were defined to assess tomato response to abiotic factors due to the complexity of stress response mechanisms. For example, Kazmi et al. (2012a, b) used seed quality traits to identify QTLs associated with tomato germination capacity under WD, CS, SS, and HT stress. They identified no less than 90 seed quality QTLs under stress conditions. Physiological parameters under WD and nitrogen-deficiency conditions were mapped in sub-NILs (Arms et al. 2016) and 130 F10 RILs (Asins et al. 2017) populations, respectively. Metabolite variation in tomato seeds under SS was studied by Rosental et al. (2016) and several QTLs were identified in 72 ILs derived from the introgression of chromosome fragments of *S. pennellii* LA716 into the domesticated tomato cultivar M82. A recent study used gene expression data under WD and control conditions and identified some WD interactive eQTLs (Albert et al. 2018). This approach permitted the distinction between *cis* and *trans* regulatory eQTL clarifying the patterns of expression regulation in tomato under WD leading to genotype-by-environment interaction. Combining expression data with QTL analysis thus helped to identify candidate stress-response genes and could be useful for the optimal choice of genetic markers to conduct MAS for stress adaptation.

However, the majority of the studies used agronomic traits instead of physiological parameters or metabolic traits to evaluate the impact of abiotic stress. This has led to the definition of different stress indexes according to breeding objectives (Table 2.4); thus QTL identified for such stress index could be directly used in breeding programs.

Until now, most QTL studies on tomato were conducted on single stress evaluation, achieving a better characterization of genetic loci involved in tomato response to a given abiotic stress. Further studies should target genomic regions that interfere in response to stress combinations. Few examples of such studies are available in plants (Davila Olivas et al. 2017).

Table 2.4 QTL studies on tomato abiotic stress published during the last decade. For each study, the number of genotypes analyzed, the population cross-design, and the number and type of markers used are displayed. The columns "Stress treatment" and "Stress period" present the level of stress applied and the period on which stress was applied. The column "Phenotypes" highlights the phenotypic traits that were evaluated to conduct the QTL/association analysis. The phenotypic traits usually correspond to different traits: Seed quality (germination ability); Fruit quality (SSC, Vitamin C, pH, firmness, organic acids); Plant architecture and vegetative growth (diameter, leaf length, height, dry matter content, specific leaf area, biomass); Phenology (flowering, ripening time); Productivity (yield, fruit weight, number of fruits); Physiological traits (WUE); Model parameters (Maximum cell wall extensibility, membrane conductivity, sugar active uptake, membrane reflection, Pedicel conductivity, soluble sugar concentration, fruit dry weight, fruit water content, xylem conductivity)

Treatment	Number of individuals	Marker types	Stress treatment	Stress period	Cross-design	Phenotypes	Number of QTLs	Reference
Cold stress (CS)								
CS	83 RILs	865 SNP	Cold stress (12 °C)	Seed germination	Bi-parental (Interspecific)	Seed quality	12 QTLs	Kazmi et al. (2012)
CS	146 RILs	120 SSR	Cold stress (11 °C)	Seed germination	Bi-parental (Interspecific)	Germinatin ratio	5 QTLs	Liu et al. (2016a, b)
CS	146 RILs	120 SSR	2 °C for 48 h	4–5 true leaves	Bi-parental (Interspecific)	Chilling injuries	9 QTLs	Liu et al. (2016a, b)
Hight temperature stress (HT)								
HT	192 F2	106 AFLP markers	Minimal/Maximal T° > 25 °C/40 °C	Transplanting—end of the experiment	Bi-parental (Intraspecific)	Fruit set	6 QTLs	Grilli et al. (2007)
HT	160 F2	62 RAPD, ISSR and AFLP markers	Day/Night T° = 37.2 °C/24.7 °C	All growing season	Bi-parental (Interspecific)	Yield; Fruit quality; Reproductive traits	21 QTLs	Lin et al. (2010)

(continued)

Table 2.4 (continued)

Treatment	Number of individuals	Marker types	Stress treatment	Stress period	Cross-design	Phenotypes	Number of QTLs	Reference
HT	83 RILs	865 SNP	Heat stress (35–36 °C)	Seed germination	Bi-parental (Interspecific)	Seed quality	16 QTLs	Kazmi et al. (2012)
HT	180 F2	96 SNP	Day/Night T° = 31 °C/25 °C	From 1st inflorescences appearance	Bi-parental (Intraspecific)	Reproductive traits	13 QTLs	Xu et al. (2017a, b)
HT	98 F8 RILs	727 SNP	37 °C	Seed germination	Bi-parental (Interspecific)	Thermo-tolerance, Thermo-inhibition, Thermo-dormancy	9 QTLs	Geshnizjani et al. (2018)
Salinity stress (SS)								
SS	123 RILs	156 SSR, SCAR markers	125 mM NaCl	15 days after transplanting to the end of the experiment	Bi-parental (Interspecific)	Rootstock induced physiological parameters; Vegetative growth	57 QTLs	Asins et al. (2010)
SS	52 ILs	!!	150 mM NaCl	21 days from the seven true leaf stage	Bi-parental (Interspecific)	Plant architecture; antioxidant content	71 QTLs	Frary et al. (2010)

(continued)

Table 2.4 (continued)

Treatment	Number of individuals	Marker types	Stress treatment	Stress period	Cross-design	Phenotypes	Number of QTLs	Reference
SS	52 ILs	!!	150 mM NaCl	15 days of treatment	Bi-parental (Interspecific)	Plant architecture; Vegetative growth	225 QTLs	Frary et al. (2011)
SS	78 ILs	!!	700 mM $NaCl^+$ 70 mM $CaCl_2$	4 days after transplanting	Bi-parental (Interspecific)	Survival performance	4 QTLs	Li et al. (2011)
SS	90 ILs	!!	700 mM $NaCl^+$ 70 mM $CaCl_2$	4 days after transplanting	Bi-parental (Interspecific)	Survival performance	6 QTLs	Li et al. (2011)
SS	100 RILs	134 SSR, SCAR markers	75 mM NaCl	15 days after transplanting to the end of the experiment	Bi-parental (Interspecific)	Rootstock induced physiological parameters; Vegetative growth	2 QTLs	Asins et al. (2010)
SS	83 RILs	865 SNP	Two levels of SS (−0.3 and −0.5 MPa NaCl)	Seed germination	Bi-parental (Interspecific)	Seed quality	32 (26) QTLs	Kazmi et al. (2012)
SS	124 RILs	2059 SNPs	8.94 dS/m	10 days after the transplanting	Bi-parental (Interspecific)	Yield; Fruit quality; Biomass	54 QTLs	Asins et al. (2015)

(continued)

Table 2.4 (continued)

Treatment	Number of individuals	Marker types	Stress treatment	Stress period	Cross-design	Phenotypes	Number of QTLs	Reference
SS	72 ILs	!!	EC = 6 dS/m	Planting—end of the experiment	Bi-parental (Interspecific)	Seed weight; Seed Germination; Metabolites	131 QTLs	Rosental et al. (2016)
SS	253 MAGIC RILs	1345 SNP	Two levels of SS (Ec = 3.7 dS/m^{-1} and Ec = 6.5 dS/m^{-1})	Transplanting—end of the experiment	MAGIC (Intraspecific)	Fruit quality; Plant architecture and vegetative growth; Phenology; Productivity	35 QTLs	Diouf et al. (2018)
Water deficit stress (WD)								
WD	75 ILs	!!	WD (30 m^3 of water irrigation for 1000 m^2)	Transplanting—end of the experiment	Introgression Line (Interspecific)	Fruit quality; Plant architecture and vegetative growth; Productivity	114 QTL	Gur et al. (2011)
WD	83 RILs	865 SNP	Two levels of Osmotic stress (−0.3 and −0.5 MPa PEG)	Seed germination	Bi-parental (Interspecific)	Seed quality	23 (19) QTLs	Kazmi et al. (2012)

(continued)

Table 2.4 (continued)

Treatment	Number of individuals	Marker types	Stress treatment	Stress period	Cross-design	Phenotypes	Number of QTLs	Reference
WD	119 RILs	679 SNP	WD (40% ETP)	Transplanting—end of the experiment	Bi-parental (Intraspecific)	Fruit quality; Plant architecture and vegetative growth; Phenology; Productivity	36 QTL	Albert et al. (2016a)
WD	141 small-fruit accessions	6100 SNPs	WD (40% ETP)	Transplanting—end of the experiment	GWAS-panel	Fruit quality; Plant architecture and vegetative growth; Phenology; Productivity	100 QTLs	Albert et al. (2016b)
WD	18 sub-NILs	10 markers (SNP; SCAR; CAP)	WD (33%ETP)	Transplanting—end of the experiment	Near-Introgression Line (Interspecific)	Physiological traits; Plant architecture	2 QTLs regions	Arms et al. (2016)
WD	117 F7 RILs	501 SNP	WD (49% ETP)	Transplanting—end of the experiment	Bi-parental (Intraspecific)	Model parameters	8 QTLs	Constantinescu et al. (2016)

(continued)

Table 2.4 (continued)

Treatment	Number of individuals	Marker types	Stress treatment	Stress period	Cross-design	Phenotypes	Number of QTLs	Reference
WD	241 MAGIC RILs	1345 SNP	WD (50% ETP)	Transplanting—end of the experiment	MAGIC (Intraspecific)	Fruit quality; Plant architecture and vegetative growth; Phenology; Productivity	22 QTLs	Diouf et al. (2018)
WD	124 RILs	501 SNP	WD (60% ETP)	Transplanting—end of the experiment	Bi-parental (Intraspecific)	Fruit quality; Plant architecture and vegetative growth; Phenology; Productivity	23 QTLs	Albert et al. (2018)
WD	124 RILs	501 SNP	WD (60% ETP)	Transplanting—end of the experiment	Bi-parental (Intraspecific)	Gene expression level for 274 genes	103 eQTL	Albert et al. (2018)
Other abiotic stress								
Oxidative stress	83 RILs	865 SNP	Oxidative stress (300 mm H_2O_2)	Seed germination	Bi-parental (Interspecific)	Seed quality	17 QTLs	Kazmi et al. (2012)
N-deficiency	130 F10 lines	1899 SNP	N-deficiency (NH_4^+: 0.1 mM and NO_3^-: 1 mM)	Transplanting—1st truss fruit set	Bi-parental (Interspecific)	Vegetative growth, Leaf nitrogen content; Xylème sap hormone content	40 QTLs	Asins et al. (2017)

Genotype-by-environment (GxE) interaction usually occurs in cultivated crops exposed to abiotic stresses. Two strategies are commonly adopted by breeders to deal with GxE: (i) developing some elite cultivars for a specific targeted environment or (ii) breeding stable cultivars for a wide range of environmental conditions. The first strategy will allow to reach high yield in predictable environments (likely controlled environments) while the second strategy will be more efficient for reducing at an optimized level, the yield decrease in unpredictable environments. This has led plant geneticists into the question of genetic control of phenotypic plasticity related to GxE phenomenon. Some studies addressed this question in major crop species and identified different plasticity QTLs. Kusmec et al. (2017), for example, suggested that in maize, genes controlling plasticity for different environments are in majority distinct from genes controlling mean trait variation, assuming a possible co-selection for stability and yield performance concurrently. In tomato, plasticity QTLs were also identified in intraspecific populations under WD and SS conditions (Albert et al. 2016a; Diouf et al. 2018). Extending the environmental range to different stress conditions could be a way to reliably identify multi-stress-response genes that would be useful in the task of breeding climate-smart tomato.

2.3.9.2 Grafting as a Defense Against Stresses

For many plant species specially vegetables and fruit trees, grafting has been considered as a solution to manage soil-borne disease and to improve crop response to a variety of abiotic stresses (King et al. 2010). For stress induced by extreme soil conditions, grafting elite cultivars onto genetic resistant rootstocks is an attractive alternative to introgression from wild resources due to the side effects of linkage drag and the polygenic nature of abiotic stress tolerance. However, grafting requires paying specific attention to the scion x rootstock combination in order to achieve better performance. In tomato, interactions between the scion and the rootstock were detected in different grating operations with alteration in fruit quality components, plant vigor, plant hormonal status, and final yield (Kyriacou et al. 2017). This highlights the necessity to test different combinations of scion-rootstocks in one hand, and in the other hand to have a better understanding of how grafting impacts the targeted breeding traits for efficient utilization of rootstocks under stressful environments.

Different tomato rootstock populations were developed and characterized accordingly. This involves populations generated from interspecific crosses between a cherry tomato accession and two wild relatives from *S. pimpinellifolium* and *S. cheesmaniae* (Estañ et al. 2009). These populations were studied under salinity (Albacete et al. 2009; Asins et al. 2010, 2015, 2013) and N-deficiency stress conditions (Asins et al. 2017). They revealed that grafting could induce variation in leaf hormonal content and ion concentrations correlated to vegetative growth and yield under salinity. The effect mediated by rootstock under salinity has a polygenic nature and is controlled by different QTLs among which one, located on chromosome 7, was related to two HTK candidate genes, involved in ion transport and cell homeostasis regulation. However, while grafting under salinity presents a promising approach to maintain or

increase tomato yield, some drawbacks were recorded concerning higher incidence of BER and delayed fruit ripening.

The hormonal status changes induced by rootstock was also shown as being potentially exploitable to increase tomato WUE (Cantero-Navarro et al. 2016). More generally, Nawaz et al. (2016) reviewed the effect of grafting on ion accumulation within horticultural crops highlighting the need for deeper characterization of rootstock x scion x environment interaction both at phenotype and genetic levels for effective utilization of grafting as a technique to manage extreme soil conditions for crops.

Besides the direct use of genetic control of pests and pathogens, grafting susceptible cultivars onto selected vigorous rootstocks may counteract soil-borne biotic stresses as well as abiotic stresses. Grafting was also proposed for improving virus resistance by enhancing RNA-silencing (Spano et al. 2015). A great challenge is consequently to breed for rootstocks that can withstand combined biotic and abiotic stresses.

2.3.10 Omic Approaches

2.3.10.1 Metabolome Analyses

Metabolomics has an important role to play in characterization of natural diversity in tomato (Schauer et al. 2005; Fernie et al. 2011). Metabolome analysis can be done in a targeted way to better characterize known metabolites (Tieman et al. 2006) or untargeted manner to identify new metabolites (Tikunov et al. 2005). As well, it can boost the biochemical understanding of fruit content and be an enhancer for quality breeding (Fernie and Schauer 2009; Allwood et al. 2011). Metabolome analyses were used to analyze fruit composition at a high-throughput level. Metabolite QTL (mQTL) has been identified for non-volatiles metabolites like sugars, pigments, or volatiles compounds (Bovy et al. 2007; Klee 2010, 2013; Klee and Tieman 2018). This was done on several interspecific populations, notably on *S. pennelli* (Alseek et al. 2015, 2017) and *S. chmielewskii* (Do et al. 2010; Ballester et al. 2016) introgression lines and intraspecific crosses (Saliba-Colombani et al. 2001; Causse et al. 2002; Zanor et al. 2009). The interaction between the tomato plant and thrips was also studied by metabolome profiling (Mirnezhad et al. 2010).

2.3.10.2 Transcriptome Analyses for EQTL Mapping

Several studies analyzed the transcriptome changes along with fruit development (Pattison et al. 2015; Giovanonni et al. 2017; Shinozaki et al. 2018) revealing key changes in gene expression during the different stages. Analysis of the genetic control of such variations in segregating populations was also performed (Ranjan

et al. 2016; Coneva et al. 2017). Characterizing the natural diversity of gene expression across environments is also an important step in understanding genotype-by-environment interactions. Albert et al. (2018) identified some eQTL in response to water stress and showed the large differences between the transcriptome of leaf and fruit under well irrigated and water stress conditions. The authors also studied allele-specific expression (ASE) in the F1 hybrid

To reveal genes deviating from the 1/1 allele ratio expected and showed a large range of genes whose variation exhibited significant ASE-by-watering regime interaction, among which ~80% presented a response to water deficit mediated through a majority of transacting.

2.3.10.3 Multi-omic Approach

Combining metabolome and transcriptome may give clues about the genetic control of fruit composition as underlined by Prudent et al. (2011). Zhu et al. (2018) performed a multi-omic study by integrating data of the genomes, transcriptomes, and metabolomes. Up to 3,526 significant associations were identified for 514 metabolites and 351 of them were associated with unknown metabolites. Correlation analysis between genomes and transcriptomes identified a total of 2,566 cis-eQTL and 93,587 trans-eQTL. Rigorous multiple correction tests between transcriptomes and metabolomes identified 232,934 expression-metabolite correlations involving 820 chemicals and 9,150 genes. By integrating these three groups, a total of 13,361 triple relationships (metabolite-SNP-gene) were further identified, including 371 metabolites, 970 SNPs, and 535 genes. Selection analysis discovered 168 domestication sweeps and 151 improvement sweeps, representing 7.85% and 8.19% of the tomato genome, respectively. A total of 4,095 and 4,547 genes were located within the identified domestication and improvement sweeps. In addition, a total of 46 steroidal glycoalkaloids was identified and five significant associations were located within domestication or improvement sweeps. They also showed that the introgression of resistance genes also introduced significant differences in some metabolites.

2.3.10.4 MiRNA and Epigenetic Modifications

Epigenome is the complete set of epigenetic marks at every genomic position in a given cell at a given time (Taudt et al. 2016). These marks fall into six categories, including DNA modifications, histone modifications, chromatin variants, nucleosome occupancy, RNA modifications, non-coding RNAs, chromatin domains, and interactions (Stricker et al. 2017). Technological advances nowadays make it possible to achieve high-resolution measurements of epigenome variation at a genome-wide scale and great achievements have been made in human, rat, yeast, maize, tomato, Arabidopsis, and soybeans (Taudt et al. 2016; Giovannoni et al. 2017).

Most of epigenome studies in tomato focused on the molecular regulations of fruit ripening and development (Gallusci et al. 2016; Giovannoni et al. 2017).

Among these, histone posttranslational modifications play an important role, which include phosphorylation, methylation, acetylationand mono-ubiquitination of lysine residues (Berr et al. 2011). In Arabidopsis, histone posttranslational modifications are involved in many aspects of plant development and stress adaptation (Ahmad et al. 2010; Mirouze and Paszkowski, 2011).In tomato, at least nine DNA methyltransferases and four DNA demethylases have been identified (Gallusci et al. 2016). Expression patterns of different histone modifiers in some fresh fruits have also been identified, such as histone deacetylases, histone acetyltransferase, and histone methyltransferases (Gallusci et al. 2016). Repression of tomato Polycomb repressive complex 2 (PRC2) components *SlEZ1* altered flower and fruit morphology (How Kit et al. 2010) and *SlEZ2* altered fruit morphology, such as texture, color, and storability (Boureau et al. 2016). These results demonstrated that epigenetic regulations are important for many biological processes.

Very few phenotypes have been associated with epi-mutations. Manning et al. (2006) identified a naturally occurring methylation epigenetic mutation in the SBP-box promoter residing at the colorless non-ripening (*Cnr*) locus, a major component in the regulatory network controlling tomato fruit ripening (Eriksson et al. 2004). Quadrana et al. (2014) identified an epi-mutation responsible of the variation in vitamin E in the fruit. In order to determine whether the process of tomato fruit ripening involves epigenetic remodeling, Zhong et al. (2013) found that tomato ripen prematurely under methyltransferase inhibitor 5-azacytidine. Up to 52,095 differentially methylated regions were identified, representing 1% of the tomato genome. In particular, demethylation regions were identified in the promoter regions of numerous ripening genes. In addition, the epigenome status was not static during tomato fruit ripening (Zhong et al. 2013). Shinozaki et al. (2018) performed a high-resolution spatio-temporal transcriptome mapping during tomato fruit development and ripening. Some tissue-specific ripening-associated genes were identified, such as SlDML2. Together with other analyses, these results indicate that spatio-temporal methylations play an important role during tomato fruit development and ripening (Shinozaki et al. 2018).

Lü et al. (2018) investigated the functional elements of seven climacteric fruit species (apple, banana, melon, papaya, peach, pear, and tomato) and four non-climacteric fleshy fruit species (cucumber, grape, strawberry, and watermelon). By analyzing 361 transcriptome, 71 accessible chromatin, 147 histone, and 45 DNA methylation profiles from the fruit ENCODE data, three types of transcriptional feedback circuits were identified controlling ethylene-dependent fruit ripening (Lü et al. 2018). In particular, H3K27me3, associated with silencing of the flowering regulator FLOWERING LOCUS C and floral homeotic gene AGAMOUS (He 2012), played a conserved role in dry and ethylene-independent fruits by restricting ripening genes and their orthologs.

MicroRNA (miRNAs) is another type of epigenetic regulation. miRNAs are a class of 20- to 24-nucleotide non-coding endogenous small RNAs that are important in transcriptional or post-transcriptional regulation by transcript cleavage and translation repression (Chen 2005, 2009; Rogers and Chen 2013; Sanei and Chen 2015).

miRNAs are encoded by miRNA genes, which contain the TATA-box motif and transcription factor binding motifs, and are regulated by general specific transcription factors (Xie et al. 2005; Megraw et al. 2006; Rogers and Chen 2013; Yu et al. 2017). miRNAs play an important role in many biological processes, including physiological, developmental, defense, and environmental changes both in humans (Calin and Croce 2006; Mendell and Olson 2012; Cui et al. 2017b; Hill and Tran 2018), animals (Ambros 2004; Rajewsky 2006; Grimson et al. 2008) and plants (Rogers and Chen 2013; Won et al. 2014; Sanei and Chen 2015; Cui et al. 2017a; You et al. 2017; Yu et al. 2017). Some regulatory mechanisms of the core components of the dicing complex, such as DICER-LIKE1 (DCL1) and HYPONASTIC LEAVES1 (HYL1) have been uncovered (Manavella et al. 2012; Cho et al. 2014; Zhang et al. 2017). Proteins promoting pre-miRNA processing and reducing miRNA levels have also been identified, such as CAP-BINDING PROTEIN 80 (CBP80), CAP-BINDING PROTEIN 20 (CBP20), STABILIZED1 (STA1), and others (Gonatopoulos-Pournatzis and Cowling 2015; Yu et al. 2017). Some proteins could reduce the accumulation of both mature pre-miRNA and mature miRNA, such as CDC5, NOT2, Elongator, and DDL (Yu et al. 2008; Wang et al. 2013a, b; Zhang et al. 2013; Fang et al. 2015). Though many processes involved in miRNA biogenesis, degradation and activity have been discovered, our knowledge regarding the subcellular locations of these processes is still largely unknown (Yu et al. 2017).

During the tomato genome sequencing, a total of 96 conserved miRNA genes were predicted. Among them, 34 miRNA have been identified and 10 are highly conserved in both tomato and potato (The Tomato Genome Consortium 2012). Several studies focused on the characterizations of miRNAs in tomato during fruit development (Moxon et al. 2008; Zuo et al. 2012; Gao et al. 2015). The dominant sRNAs were 21- to 24-nt sRNAs (Mohorianu et al. 2011; Zuo et al. 2012; Gao et al. 2015). Many ripening-associated gene transcription factors were regulated by certain miRNA families, such as miR156/157, miR159, miR160/167, miR164, miR171, and miR172 families (Moxon et al. 2008; Karlova et al. 2013; Zuo et al. 2013). miRNA precursor genes are also regulated by many transacting factors (Rogers and Chen 2013). Ethylene might be involved in the regulation of miRNA and also their corresponding precursor genes, such as TAS3-mRNA, miR156, miR159, miR160, miR164, miR171, miR172, miR390, miR396, miR4376, and miR5301 (Gao et al. 2015). RIN (ripening inhibitor) regulates tomato fruit ripening-related genes through of the post-transcriptional regulations of related genes via miRNA and ethylene. In addition, the ethylene can also regulate miRNA by modulating the abundance of mRNA (Gao et al. 2015). miRNAs specifically induced in response to biotic or abiotic stresses have also been identified and could be interesting targets for tomato adaptation (Liu et al. 2017). Though epigenome regulation is important during fresh fruit development and ripening, additional investigations about epigenome dynamics during fruit maturation and ripening or under environmental stresses are still needed (Giovannoni et al. 2017).

Table 2.5 Main databases useful for tomato genetics and genomics

Name	Address	Characteristics
Solanaceae Genome Network (SGN)	https://solgenomics.net	Central hub for sol genomics (genome sequences, loci, phenotypes …
Tomato Genetic Resource Center (TGRC)	https://tgrc.ucdavis.edu/	Charles Rick Tomato Genetic Resource Collection in UC Davis
Tomatoma	http://tomatoma.nbrp.jp/	Microtom mutants and genome archive
Mibase Tomato DB	http://www.kazusa.or.jp/jsol/microtom	Microtom genomic resources
SolCAP	http://solcap.msu.edu/	SNP, genotype and phenotypes
Tomato Expression Database	http://ted.bti.cornell.edu/	Gene expression analysis results
Tomato Expression Atlas	http://tea.solgenomics.net/	High-resolution map of gene expression
Tomexpress	http://tomexpress.toulouse.inra.fr/	RNAseq data
Tomato EFP browser	http://bar.utoronto.ca/efp_tomato	Tomato gene expression viewer
Solcyc	http://solcyc.solgenomics.net/	Pathway/genome DB

2.3.11 Databases

Databases are essential to access the wide range of data produced and shared on tomato. Tomato community has benefited for years of the will to gather genetic and later genomic data into one single free access database, known as Solanaceae Genome Network, as the resource concerns several Solanaceae species. Since the first RFLP genetic map, the database hosts information about markers, genes, and QTL and now a genome browser where several genomes and SNP can be found. Several other databases can be useful to tomato geneticists. They describe genetic resources and mutant collections or information about gene expression (Table 2.5).

2.4 Breeding for Smart Tomato

2.4.1 Traditional Breeding

Tomato is a self-pollinated crop. The first varieties were landraces and the intensive breeding started in the 1930s in the USA. As a self-pollinated crop, for years, tomato

has been bred through a combination of pedigree and backcross selection. Very early, introgressions from wild species were proposed to introduce disease resistances but also to improve fruit firmness and other fruit quality traits (Bai and Lindhout 2007). Recurrent selection (successive rounds of selection and intercrossing of the best individuals) also proved efficient to simultaneously increase fruit sugar content and fruit size and break the negative relationship between both traits (Causse et al. 2007a, b).

Although tomato exhibits a low heterosis for yield, F_1 hybrid varieties progressively replaced the pure lines since the 1970s. This was first shown to be interesting for fruit shape and size homogeneity and then for combining several dominant resistance genes. Today F1 hybrids combine 6 to 8 disease resistance genes. For the production of F1 seeds, a set of nuclear recessive male sterility genes have been described, but are not used for a commercial purpose. The use of a functional male sterility gene, controlled by the positional sterile mutation (*ps2*) whose anthers do not naturally open, has been proposed (Atanassova 1999). Nevertheless, due to the difficulty of carrying sterility genes along with the selection schemes and to the rapid turnover of tomato cultivars, F1 hybrids are more frequently produced by hand pollination, in countries with low labor cost.

2.4.2 Marker-Assisted Selection

Many important loci have been mapped and tagged with molecular markers. Marker-assisted selection (MAS) allows breeders to follow genomic regions involved in the expression of traits of interest. The efficiency and complexity of MAS depend on the genetic nature of the trait (monogenic or polygenic). For monogenic traits, marker-assisted backcross (MABC) is the most straightforward strategy, whereas for polygenic traits, various strategies are available.

2.4.2.1 Marker-Assisted Backcross for Monogenic Traits

The principle of MABC for a single gene is simple. First, molecular markers tightly linked to the target gene are identified, allowing the efficient detection of the presence of the introgressed gene ("foreground selection"). Other markers may be also used in order to accelerate the return to the recipient parent genotype at other loci ("background selection"). Background selection is based not only on markers located on the chromosomes carrying the gene to introgress (carrier chromosome), but also on other chromosomes. Markers devoted to background selection on a carrier chromosome allow the identification of individuals for which recombination events took place on one or both sides of the gene, in order to reduce the length of the donor type segment of genome dragged along with the gene (Young and Tanksley 1989). In three generations of MABC, isogenicity is higher than that obtained by classical methods. By comparison, traditional approach would require approximately two more generations

to obtain such an isogenicity (Hospital et al. 1992). Many important genes have been mapped or even cloned and specific markers for favorable alleles developed (Rothan et al. 2019 for a recent review). Today, tomato breeders use molecular markers for the introgression of several monogenic traits such as disease resistances or fruit-specific traits. The reduction of the cost of genotyping allows today the screening of a large number of plants to accelerate the selection process.

2.4.2.2 Marker-Assisted Selection for QTLs

Traits showing a quantitative variation are usually controlled by several QTLs, each with a different individual effect. Due to the genetic complexity of such traits, several QTLs with limited effects must be simultaneously manipulated. Depending on their number, the nature and range of their effect, and the origin of favorable alleles, different MAS strategies were proposed.

As for monogenic traits, MABC is the most effective strategy when a small number of QTLs, coming all from the same parent, must be transferred into an elite line. Hospital and Charcosset (1997) determined the optimal number and positions of the markers needed to control the QTLs during the foreground selection step and the maximum possible number of QTLs that could be simultaneously monitored with realistic population sizes (a few hundred individuals). On average, using at least three markers per QTL allows a good control over several generations, providing a low risk to have the donor type alleles at the markers without having the desired genotype at the QTL. However, as the minimum number of individuals that should be genotyped at each generation depends on (i) the confidence interval length, (ii) the number of markers, and (iii) the number of QTLs, it seems illusive to transfer more than four or five QTLs with this simultaneous design unless a very large population can be considered, or the precision of the QTL location is very high.

After the identification of QTL for fruit quality traits (Saliba-Colombani et al. 2001; Causse et al. 2001), several clusters of QTLs were identified. As most of the favorable alleles for quality improvement came from the cherry tomato parental line, a MABC scheme has then been set up in order to transfer the five regions of the cherry tomato genome with the largest effects on fruit quality into three recurrent lines (Lecomte et al. 2004b). The population size allowed a successful transfer of the five segments into each recurrent line, and the MAS scheme allowed reducing the proportion of donor genome on the non-carrier chromosomes under the level expected without selection. Plants carrying from one to five QTLs were selected in order to study their individual or combined effects. Most of the QTLs were recovered in lines carrying one introgression region and new QTLs were detected (Causse et al. 2007a, b). Introgressed lines had improved fruit quality, in comparison to parental lines, promising a potential improvement. Nevertheless, fruit weight in these genotypes was always lower than expected due to the effect of unexpected QTLs, whose effect was masked in the RIL population, suggesting that negative alleles at fruit weight QTLs were not initially detected.

2.4.2.3 Advanced Backcross for the Simultaneous Discovery and Transfer of New Alleles

The advanced backcross QTL analysis is another strategy tailored for the simultaneous discovery and transfer of valuable QTL alleles from unadapted donor lines into established elite inbred lines (Tanksley and Nelson 1996). The QTL analysis is delayed until an advanced generation (BC_3 or BC_4), while negative selection is performed to reduce the frequency of deleterious donor alleles during the preliminary steps. The use of BC_3/BC_4 populations reduces linkage drag by reducing the size of introgressed fragments, limits epistatic effects, and decreases the amount of time later needed to develop near-isogenic lines carrying the QTL (Fulton et al. 1997). Tanksley and colleagues have applied this strategy for screening positive alleles in 5 wild species, *S. pimpinellifolium* (Tanksley et al. 1996), *S. habrochaites* (Bernacchi et al. 1998a), *S. peruvianum* (Fulton et al. 1997), *S. pennellii* (Eshed et al. 1996) et *S. parviflorum* (Fulton et al. 2000). They identified a number of important transgressions potentially useful for processing tomato and demonstrated that beneficial alleles could be identified in unadapted germplasm and simultaneously transferred into elite cultivars, thus exploiting the hidden value of exotic germplasm (Bernacchi et al. 1998b, Tanksley and Nelson 1996).

2.4.2.4 Pyramidal Design

When the number of QTLs to introgress becomes important, Hospital and Charcosset (1997) proposed to use a pyramidal design. QTLs are first monitored one by one by MABC, to benefit from higher background selection intensity, and then the selected individuals are intercrossed, to cumulate favorable alleles at the QTLs in the same genotype. When favorable alleles come from different sources, van Berloo and Stam (1998) proposed an index method to select among recombinant inbred lines those to be crossed and to obtain a single genotype containing as many favorable quantitative trait alleles as possible. Plants showing the optimal index are crossed together. This strategy was shown efficient to obtain transgression in offspring populations of *Arabidopsis* (van Berloo and Stam 1999).

The benefit of MAS for QTL pyramiding was shown but limited by the number of QTLs easily managed (Lecomte et al. 2004b; Gur and Zamir2015; Sacco et al. 2013). This can be overcome by fine mapping experiment and/or validating the QTL effect in other backgrounds (Lecomte et al. 2004a). Today SNP availability and genomic selection open new ways to marker-assisted selection for quantitative traits.

2.4.2.5 Breeding for Resistance to Pests and Pathogens

Despite decades of conventional breeding and phenotypic selection, there are still a large number of pests and pathogens that make tomato production challenging

in various parts of the world. It is why the most prominent issue of tomato breeding remains pest and pathogen resistance. Current advances in tomato genetics and genomics can be combined with conventional plant breeding methods to introgress resistance loci or genes and expedite the breeding process.

Phenotypic (e.g., sensitivity to the Fenthion insecticide linked to resistance to *Pseudomomas syringae* pv. *Tomato* Laterrot and Moretti 1989), enzymatic (e.g., Aps-1^1 linked to rootknot nematode resistance Aarts et al. 1991, Messeguer et al. 1991) and DNA markers tightly linked to resistance loci have long been used for MAS to incorporate resistance loci in new tomato cultivars. MAS is valuable for increasing the efficiency of selection, particularly when it is difficult to perform disease resistance assay, for instance with quarantine pathogens requiring controlled experimental infrastructures, and when disease resistance is controlled by recessive genes, or when genes display a weak penetrance or are strongly influenced by environment. Markers help to carry on a more efficient and precise introgression of the targeted loci, reducing the negative effects of linkage drag. MAS has also permitted to pyramid several resistance loci with other desirable traits. Because most of the resistance genes are clustered on the tomato genome, introgression of resistance traits by phenotyping selection or by using MAS with markers at both sides of the major resistance gene permitted to introgress a kind of cassettes of resistance alleles when they are in coupling linkage and to create multi-resistant cultivars. For instance, most of *Tm-*2^2 tomato cultivars hitchhiked the *Frl* gene responsible for the Fusarium crown and root rot resistance caused by FORL (Foolad and Panthee 2012). Inversely, when resistance alleles are linked in repulsion phase, breeding selection may be hindered by the difficulty to select for homozygous coupling-phase recombinant lines, as illustrated for the association of *Sw-5* and *Ph-3* (Robbins et al. 2010). Thanks to MAS, the rate of improvement has been significantly enhanced in tomato even if many challenges remain.

Nowadays, DNA markers have been made available for about 30 genes controlling single gene inherited resistance traits important for tomato breeding (https://solgenomics.net/; Foolad and Panthee 2012). DNA markers for complex inherited resistance traits are much less abundant and they have rarely been used. MAS is thus routinely employed for selecting major effect resistance genes (*I*, *I-2*, and more recently, *I-3*, *Ve*, *Mi-1.1*/*Mi1.2*, *Asc*, *Sm*, *Pto*, *Tm-*2^2, *Sw-5*) and many commercial cultivars now are resistant to *Fusarium oxysporum f. sp. lycopersici*, *Verticillium dahlia*, *Meloigogyne incognita*, *Alternaria alternata* f.sp. *lycopersici*, *Stemphyllium*, *Pseudomonas syringae* pv. *tomato*, ToMV, and TSWV. Also, markers for *Rx-3* and *Rx-4*, and for *Ty-1*, *Ty-2*, *Ty-3*, *Ty-4* are more and more used to deliver resistant cultivars to *Xanthomonas* spp. and TYLCV.

Although markers have been identified for many disease resistance in tomato, not all of them are useful because of the absence of polymorphism within breeding populations that are often based on intraspecific crosses or because markers are too far from genes or QTLs of interest permitting unwanted crossing-overs. However, advances in next-generation sequencing make possible to identify linked SNPs from which new PCR-based markers can be developed for trait association within breeding populations. The whole plant genome technologies greatly help to identify useful

markers linked to resistance traits within the wild germplasm by ecoTILLING, allele mining, or GWAS. Tomato breeders are thus now able to select the best combinations of genotypes to intercross in order to associate favorable traits and design elite ideotypes.

2.4.3 Genomic Selection

Many traits are controlled by a large number of QTLs with low effect. Both linkage mapping and GWAS have limitations in identifying and quantifying small effect and also rare QTLs or associations that are highly susceptible to environmental conditions (Crossa et al. 2017). In contrast, genomic selection (GS), which has been proposed for about two decades (Meuwissen et al. 2001; Crossa et al. 2017) uses all the genetic information from markers spread over the whole genome, such as SNPs and phenotypic data, in a training population, to predict the genetic estimated breeding values (GEBVs) of unphenotyped individuals in a test population. The main advantages of GS include cost reduction and time saving compared to phenotype-based selection (Crossa et al. 2017).

Several factors influence the accuracy of genomic prediction (GP), including the size, structure, and genetic diversity of the training population, trait heritability, the number and distribution of molecular markers, linkage disequilibrium, prediction method, and number of QTLs (Isidro et al. 2015; Spindel et al. 2015; Duangjit et al. 2016; Kooke et al. 2016; Yamamoto et al. 2016; Boison et al. 2017; Crossa et al. 2017; Minamikawa et al. 2017; Müller et al. 2017; Yamamoto et al. 2017; Crain et al. 2018; Edwards et al. 2019; Mangin et al. 2019; Sun et al. 2019). In order to improve the prediction accuracy, complex GS models were developed in order to handle different factors, such as the multi-trait and multi-environment G×E interactions (Montesinos-López et al. 2016; Fernandes et al. 2018). To date, many models for GS are available and the prediction accuracy varies according to traits and conditions (Heslot et al. 2012; Jonas and de Koning 2013; Yamamoto et al. 2016, 2017).

The first GS test in tomato was focused on a simulation-based breeding design and phenotypic prediction, where a theoretical method was proposed to apply GS to actual breeding schemes of simultaneous improvement of yield and flavor (Yamamoto et al. 2016). Briefly, 96 big-fruited tomato varieties were selected and 20 agronomic traits were measured, which can be divided into four categories, including yield, quality, physiological disorder of fruit, and others, with the broad-sense heritability ranging from 0.10 to 1.00. Seven GP models were compared, including five linear methods, Ridge regression (RR) (Endelman 2011), Bayesian Lasso (BL) (Park and Casella, 2008), extended Bayesian Lasso (EBL) (Mutshinda and Sillanpää 2010), weighted Bayesian shrinkage regression (wBSR) (Hayashi and Iwata 2010), and Bayes C (Habier et al. 2011), and two nonlinear methods, reproducing kernel Hilbert space regression (RKHS) (Gianola and van Kaam 2008) and random forest (RF) (Breiman 2001). The highest prediction accuracy for different traits varied and the accuracy of Bayes C was highest for up to eight traits, ranking the best among all models.

Some individuals with high GEBV of total fruit weight and soluble solid contents were selected as parents to simulate later generations. Simulations demonstrated that after five generations, the simulated GEBVs were comparable with parental varieties. Breeding selections of target traits could also have impact on some non-target traits. In particular, simultaneous selection for yield and flavor resulted in morphological changes, such as the increase in plant height. These results demonstrated the benefits of simulations for real breeding design.

Yamamoto et al. (2017) then used big-fruited F_1 population to construct the GS models to assess its potential for the improvement of total fruit weight and soluble solid content in a practical experiment. By testing six GS models and 10-fold cross-validation, the prediction accuracy for soluble solid content was higher than for total fruit weight. GBLUP and BL had significantly higher predictability compared to other models for soluble solid content. In contrast, RKHS and RF had significantly higher predictability compared to other linear models for total fruit weight. The authors further developed four progeny populations to predict trait segregations and demonstrated that all individuals in the four progeny populations were genetically distinct from each other but intermediate between their parental varieties. However, the genetic diversity within each population was much lower compared to the training population.

Duangjit et al. (2016) investigated the impacts of some key factors on the efficiency of GP, including the size of training population, the number and density of SNPs, and individual relatedness. Based on the analysis of 163 tomato accessions, the optimal size of the training population was 122. The prediction accuracy also increased with the increase of marker density and number, but weakly. Individual relatedness also influenced the prediction accuracy, and predictions were better in closer individual relatedness. However, there are some limitations in this study: (1) it only tested the ridge regression best linear unbiased prediction (rrBLUP) statistical model (Endelman 2011); (2) the number of SNPs was relatively small and the genomic coverage in certain genomic regions was quite limited (Zhao et al. 2019); (3) population structure existed and the number of wild accessions was quite small compared to cherry and large-fruited tomato accessions.

Most of the GS models rely on marker-based information and are unable to exploit local epistatic interactions among markers. Molecular markers can also be combined into haplotypes by combining linkage disequilibrium and linkage analysis to improve prediction accuracy (Clark 2004; Calus et al. 2008; Jiang et al. 2018), which has been recently shown especially in animals (Calus et al. 2008; Cuyabano et al. 2014, 2015a, b; Hess et al. 2017; Karimi et al. 2018). Haplotype-based genome-wide prediction models make it possible to exploit local epistatic effects inside haplotype blocks (Wang et al. 2012; de Los Campos et al. 2013; He et al. 2016; Jiang et al. 2018). The benefits of haplotype-based GS remain to be investigated in major crops (Jiang et al. 2018).

Genomic selection should permit to breed for a combination of traits related to qualitative resistance to biotic stresses as well as quantitative resistance and tolerance to biotic and abiotic stress combinations considering also the genetic architecture of yield and fruit quality-related traits. Both foreground and background

selection should promote a sustained performance under diverse changing environments. Until now, disease quantitative resistance does not seem to be actively pursued by breeders because the complex polygenic control has generally hampered a wide deployment of QTL introgression. The development of post-genomics should help to foster tomato breeding for multiple polygenic traits including multi-resistance to pests and pathogens.

2.5 Designing Ideotypes by Ecophysiological Modeling

Until the 1970s, genetic advances have favored the creation of high-yielding varieties adapted to mechanized and high-input production systems. Since the 90s, the context of global change instigates to renew the breeding goals by taking into account multiple environmental, economic, and social issues. These multidisciplinary and integrative approaches have combined genetics and ecophysiology or agronomy skills, taking into account the mechanisms linking phenotypes to genotypes, and their modulation by the environment (essentially defined by soil, climate, and pests) and cultural practices. Such approaches have allowed for a meaningful assessment of genotype-environment interactions and plant performances in terms of yield, quality, and environmental impact in current production contexts. They have also made it possible to combine genetic information (available through the emergence of genetic and genomic tools) with phenotypic traits that determine variables of agronomic interest. In this context, the notion of ideotype has progressively developed to design plants able to perform in a given production context and finally to define breeding targets. To this end, process-based predictive models have proven their efficiency to unravel the mechanisms behind genetic variability of complex traits (Reymond et al. 2003; Tardieu 2003; Quilot et al. 2005; Struik et al. 2005), to analyze Genotype x Environment x Management (GxExM) interactions (Génard et al. 2010; Bertin et al. 2010; Martre et al. 2011), or to design new ideotypes adapted to specific environments (Kropff et al. 1995; Quilot-Turion et al. 2016; Martre et al. 2015; Génard et al. 2016).

2.5.1 What Is an Ideotype?

The ideotype concept, first proposed for wheat and then extended to several domesticated crops, is "a theoretical biological model which is expected to perform or behave in a predictable manner within a defined environment" (Donald 1968). Martre et al. (2015) extended the ideotype definition, to "the combination of morphological and physiological traits (or their genetic bases) conferring to a crop a satisfying adaptation to a particular biophysical environment, crop management, and end use".

Application for breeding may be straightforward for monogenic traits such as some biotic stress resistance. For instance, Zsögöna et al. (2017) proposed to take

advantage of genome-editing techniques in order to tailor such monogenic traits in cultivated cultivars or, on the opposite, to manipulate yield-related traits in wild relatives harboring polygenic stress resistance. Things are more complicated in case of traits with polygenic basis, for which geneticist has to face major issues. One of them is the complexity of some selection targets, such as yield, quality, nitrogen use efficiency, or adaptation to water deficit. Indeed these traits result from numerous nested processes with feedback effects and therefore, they are controlled by many genes. Another issue lies in the fact that the expression of these characters also depends on the environment and farming practices. This often results in strong GxExM interactions that make genetic work and their breeding application difficult. In a first empirical approach, optimal combinations of traits adapted to one specific environment and production system could be easily designed. For extrapolation to many different contexts, process-based predictive models may play a major role as discussed below (Quilot-Turion et al. 2012; Génard et al. 2016).

2.5.2 Current Process-Based Models of Tomato for the Prediction of GxExM Interactions

The plant and its organs can be seen as complex systems in which many processes interact at different scales under the control of GxExM interactions. Process-based predictive models are formal mathematical descriptions of this system and they have the potential to mimic its complexity in interaction with the environment, by integrating processes at several organizational levels (from cell to plant). The so-called component traits, which are underlying the predicted complex traits, are characterized in terms of model parameters, which instead of the complex trait itself, may subsequently be linked to underlying genetic variations (Struik et al. 2005; Bertin et al. 2010). This usually consists in forward genetics approaches such as QTL mapping, in which one searches for co-localizations between QTL for traits and QTL for model parameters (e.g., Yin et al. 1999; Reymond et al. 2003; Quilot et al. 2005; Prudent et al. 2011; Constantinescu et al. 2016). Thus, a preliminary step is the identification of specific genotype-dependent parameters of the model in opposition to other generic parameters that do not vary among genotypes. Then each combination of genes or alleles is represented by a set of parameters and the phenotype can then be simulated in silico under various environmental and management conditions. In order to extend the range of prediction beyond known genotypes, it is necessary to estimate the values of the genotypic parameters depending on combinations of QTLs (QTL-based models), alleles, or genes (gene-based models) involved in the modeled process (Martre et al. 2015). By formalizing each individual trait as a combination of genotypic and environmental effects, the model-based approach allows to detect more QTL that tends to be more stable than traditional QTL mapping. However, up to date, only a few genotypic parameters (i.e., allelic variants) have been advantageously

introduced into simulation models of tomato (Prudent et al. 2011; Constantinescu et al. 2016).

Several process-based simulation models that predict the processes underlying fruit growth and quality are now available and allow exploring the myriad of GxExM combinations (Génard and Lescourret 2004; Bertin et al. 2010; Martre et al. 2011; Kromdijk et al. 2013). For tomato, several plant models are driven by processes of carbon assimilation and allocation among sinks according to different rules of priority (Heuvelink and Bertin 1994; Jones et al. 1991; Boote 2016; Fanwoua et al. 2013), while only a few models simulate the water transfer and accumulation. For instance, Lee (1990) considers a unidirectional and constant flux of water uptake and transpiration per unit of fruit area. Bussières (1994) developed a model of water import in tomato fruit, based on water potential gradients and resistances. Yet, only rare models of fruit growth integrate both dry matter and water accumulation within the fruit. A virtual fruit model developed for peach (Fishman and Génard 1998) has been adapted to predict processes involved in tomato fruit growth and composition (Liu et al. 2007). This model relies on a biophysical representation of one big cell, in which sugars are transported from the fruit's phloem by mass flow, diffusion, and active transport. Incoming water flows are regulated, in particular, by differences in water potential and growth is effective only when the flow balance induces a sufficient turgor pressure on the cell walls. These models have been further modified and coupled to a stem model to estimate the contribution of xylem and phloem (Hanssens et al. 2015) and evaluate the effect of crop load on fruit growth (De Swaef et al. 2014).

The Virtual Fruit model has been also combined with a structural plant model to predict water and carbon allocation within the plant architecture, as well as the induced gradients of water potential and phloem sap concentration in carbon (Baldazzi et al. 2013). Because the cell level is the elementary level for mechanistic modeling of fruit (Génard et al. 2010), a crucial issue is to model the way cell division and expansion developmentally progress (Baldazzi et al. 2012; Okello et al. 2015). The rare models of tomato fruit, which integrate cell division, cell expansion, and DNA endoreduplication, have been used to better understand the emergence of fruit size and cell distribution (Fanwoua et al. 2013; Baldazzi et al. 2017, 2019). A virtual fruit model that predicts interactions among cell growth processes would be able to integrate subcellular models (Beauvoit et al. 2018), such as the ones proposed for tomato fruit to describe metabolic shifts during fruit development (Colombié et al. 2015, 2017) and pericarp soluble sugar content based on enzyme activity and compartmentation (Beauvoit et al. 2014). Indeed, except for sugar metabolism (Prudent et al. 2011), there is still a lack of predictive models of fruit composition, which is a major issue for fruit quality. For instance, no mechanistic model predicts the main compounds involved in tomato health value, like carotenoids, polyphenols, or vitamins, which deserve further development. Such models exist for peach acidity (Lobit et al. 2003, 2006) and could be tailored to tomato.

Such integrated models centered on the fruit, integrating cellular processes and connected to a plant model open major perspectives to integrate information on the molecular control of fruit growth and composition regulations and to analyze the

effects of GxExM interactions on yield and quality (Martre et al. 2011). Indeed, integrated models are important tools to phenotype plant in silico. They do not only allow to predict plant and organ traits such as yield or fruit composition, but also to asses physiological variables that are not easily measured on large panels such as xylem and phloem fluxes, active sugar transport… (Génard et al. 2010). So, process-based models enable to better understand genetic variability and identify candidate genes. They can also assist breeders to identify the most relevant traits and appropriate developmental stages to phenotype plants, and provide necessary links between genotype and phenotype in a given environmental context (Struik et al. 2005).

2.5.3 Process-Based Models Design of Tomato Ideotypes

An important issue of simulating GxExM interactions is the in silico design of ideotypes, i.e., combinations of QTL/genes/alleles relevant to optimize fruit growth and quality under specific conditions, by multi-criteria optimization methods (Quilot-Turion et al. 2016). Therein lies the interest of process-based predictive models for developing breeding strategies.

A process-based model breeding program could break down into 3 successive steps (Fig. 2.6): the first step consists of determining the values of the genetic coefficients of the model that makes it possible to obtain the desired characters for the ideotypes (virtual phenotype), in a given context of production (for instance low water supply, plant pruning…). The second step is to assess the values of the genetic coefficients from the genetic point of view (virtual genotypes), which requires identifying the combinations of alleles associated with each genetic coefficient. The last step is either to search among the existing genotypes for those that are the closest to the ideotype defined for a given environment, or to propose breeding strategies to obtain new genotypes on the basis of these ideotypes. For this last step, process-based models can be coupled with genetic models accounting for the genetic architecture of the genetic coefficients to simulate the genotypic changes that are expected to occur during the breeding program. Quilot-Turion et al. (2016) further proposed to add genetic constraints to improve ideotype realism and to optimize directly the alleles controlling the parameters, taking into consideration pleiotropic and linkage effects. This approach enabled reproducing relationships between parameters as observed in a real progeny and could be very useful to find out the best combinations of alleles in order to improve fruit phenotype in a given environment.

Despite clear benefits and perspectives, only a few tomato ideotypes have been designed through modeling. Using a static functional structural plant model, Sarlikioti et al. (2011) looked for optimal plant architecture of greenhouse-grown tomato with respect to light absorption and photosynthesis. They concluded that an ideotype with long internodes and long and narrow leaves would improve crop photosynthesis. A second example based on the virtual fruit model of tomato described above, (Constantinescu et al. 2016) suggested that a successful strategy to maintain yield

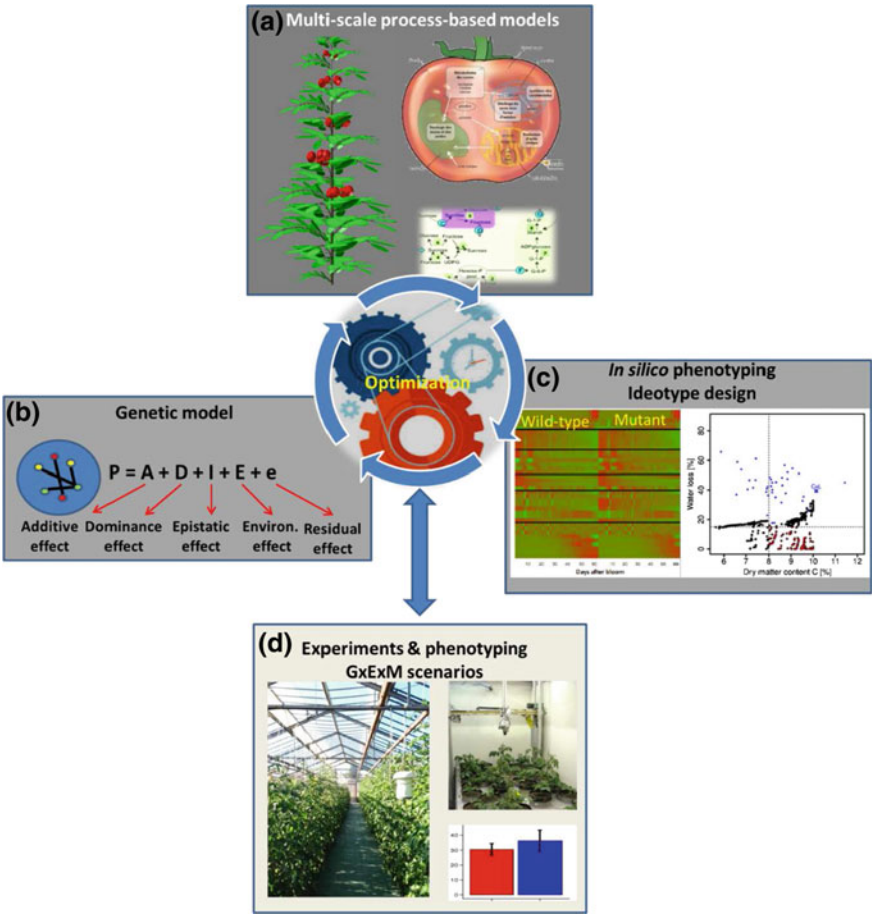

Fig. 2.6 Overall scheme of the process-based design of tomato ideotypes. Plant and organ phenotypes measured in a controlled environment or phenotyping platforms under different GxExM combinations (**d**) can be predicted by coupling process-based models that describe water and carbon fluxes in the plant, growth processes, and primary and secondary fruit metabolism (**a**). On the right, figure (**c**) illustrates the use of the coupled model for phenotyping plants and fruits and for designing ideotypes. The heatmap shows the effect on all the simulated processes of a virtual mutation controlling one genetic parameter of the model, while the plot shows the position of ideotypes generated by the model according to fruit dry matter content and fruit water loss due to water deficit. On the left (**b**), the genetic model is dependent on several effects, which control the genotypic parameters of the process-based models in (**a**). The genetic model enables to predict the genotype of ideotypes selected in (**c**). The optimization procedure applies both to estimate the genotypic parameters of the models and to design the ideotypes

and quality of large fruit genotypes under water deficit conditions could be to combine high pedicel conductance and high active uptake of sugars. Through the model calibration, the authors could identify some genotypes of the studied population, which were close to the ideotypes and thus, which may bring interesting traits and alleles for breeding plant adapted to low water supply.

As seen above, predictive models used for the design of ideotypes are expected to be highly mechanistic and detailed, therefore very complex, often combining different scales of description. Model parameters are ideally measured through adequate phenotyping, or more currently estimated through model calibration. Yet, a major difficulty is their parameterization based on extensive and heavy experiments on large genetic panels, which is rather prohibitive (Cournède et al. 2013). Similarly, the prediction of model parameters from QTL, alleles, or genes relies on a calibration step that also suffers from the relatively limited number of parameterized genotypes (Letort et al. 2008; Migault et al. 2017). Instead of measuring extensive sets of physiological traits on all genotypes of the studied population, one can select a set of genotypes that well represents the genetic diversity and then predict the parameters for the whole selection of genotypes by QTL or genomic prediction models (van Eeuwijk Fred et al. 2019). Alternatively, a representative training set of genotypes can be selected based on relevant morpho-physiological traits for estimating model parameters, as done in Constantinescu et al. (2016). From the mathematical point of view, the design of ideotypes is complex and relies on multi-objective optimization methods, which are complex due to dimensional problem (increasing number of genotypes and variables) and to the fact that ideotypes usually combine antagonistic nonlinear traits, such as yield and quality for tomato fruit. To solve the optimization problems, large panels of meta-heuristics exist, based on different algorithms that can provide satisfactory solutions in a reasonable amount of time (Ould-Sidi and Lescourret 2011). These methods can also apply to the model calibration step.

Our ability to phenotype large panels has increased in the last decades, with the emergence of high-throughput genotyping and phenotyping platforms that generate large datasets on plant morphology and physiology at high temporal and spatial resolution. The way phenotyping information can be advantageously incorporated in different classes of genotype-to-phenotype models has been recently illustrated for field crops (van Eeuwijk Fred et al. 2019). However, in the case of tomato and other horticultural plants, the range of phenotyped traits should go well beyond the traits that are routinely measured on such platforms, for instance by including fruit growth and composition alongside with plant and fruit development.

2.5.4 Prospects on the Use of Model-Based Plant Design

Model-based design of plants offers promising opportunities for both crop management and breeding of plants able to cope with different environments and to answer multiple objectives. Tomato is particularly relevant for such approach. Its sequenced

genome, the large number of genetic resources, available process-based models integrating process-networks at different organization levels, strong societal demand for high-quality fruits are all key-assets for the successful design of tomato ideotypes. Yet, some progress is still necessary. The integration of cellular and molecular levels can help refine plant models, and shed light onto the complex interplay between different spatial and temporal scales that control the traits of interest. For this, small networks of genes involved in the modeled processes might be helpful, as they could boost our capacity to link process-based model parameters to their genetic basis.

While the proof of concept is validated, it is clear that up to date, rare or no plant improvement has grounded in in silico design of ideotypes. To this end, closer collaborations among modelers, agronomists, geneticists, and breeders are necessary to combine approaches and in particular to couple process-based models and genetic models of tomato. Furthermore, the development of new process-based sub-modules predicting important tomato quality traits such as texture, carotenoid, polyphenol, and vitamin contents will be essential.

Finally, we could question the dominant paradigm according to which genetic improvement relies on gene pyramiding. Indeed, stacking multiple genes in one variety might efficiently increase multiple resistances to biotic stresses, but may fail for other traits depending on the number of genes and their genetic architecture, the nature of germplasm, etc. (Kumar et al. 2016). Instead, a new issue could be to bet on multi-genotype crops to stabilize their performances and reduce the inputs. This will require better understanding of interactions among genomes within a population.

2.6 Biotechnology and Genetic Engineering

2.6.1 A Brief History of Genetic Engineering in Tomato

According to the annual report of ISAAA (International Service for the Acquisition of Agri-biotech Applications) of 2017, 17 million farmers in 24 countries planted 189.8 million hectares biotech/GM crops. In 22 years, the planted area increased over 100 times. Nowadays there is no genetic engineered tomato available in market, whereas the first genetically engineered and commercialized food has been tomato, with a cultivar named FLAVR SAVR^{TM}, which was approved by FDA (USA) on May 18, 1994, and just 3 days later, was available in two stores. It was created by scientists in Calgene company via antisense RNA of polygalacturonase (PG), one of the most abundant proteins that had long been thought to be responsible for softening in ripe tomatoes (Kramer and Redenbaugh 1994). FLAVR SAVR^{TM} showed 99% decrease of PG protein and significant decrease in softening during storage, and increased resistance to fungi, which normally infects ripe fruits, thus providing a longer shelf life. Scientists expected that this tomato could be vine-ripened for enhanced flavor, and still suitable for the traditional distribution system (Kramer et al. 1992). At the same year, Zeneca commercialized a tomato puree made from tomatoes silenced PG with sense gene, with improved viscosity and flavor, and reduced waste (Grierson

Table 2.6 Transgenic tomato varieties approved for commercialization, reproduced from Gerszberg et al (2015)

Event	Developer	Traits	Year	Approved for	Country
FLAVR SAVR	Calgene	Delayed softening(developed by additional PG gene expressed)	1994	All uses in USA; Japan, and Mexico for feed and for environment	USA
1345-4	DNA Plant Technology Corporation	Delayed ripening (developed by a truncated aminocyclopropane cyclase synthase gene)	1994	All uses in USA; food in Canada and Mexico	USA
Da,V,F tomato	Zeneca Seeds	Delayed ripening (developed by additional PG gene expressed)	1994	All uses in USA; food in Canada and Mexico	USA
8338	Monsanto Company	Delayed ripening (developed by introduction of 1-aminocyclopropane-1-carboxylic acid deaminase (accd) gene)	1995	All uses in USA	USA
351 N	Agritope	Delayed ripening (developed by introduction the S-adenosylmethionine hydrolase (SAMK) gene)	1995	All uses in USA	China
Huafan No 1	Huazhong Agricultural University	Delayed ripening (developed by introduction antisense EFE gene)	1996	Data not available	China
5345	Monsanto Company	Insect resistant (developed by introduction of one cry1Ac gene)	1997	All uses in USA; food in Canada	USA
PK-TM8805R (8805R)	Beijing University	Delayed ripening	1999	Food, feed, cultivation in China	China

2016). The success was not as expected. FLAVR SAVR was removed from the market in 1999. Later a dozen of genetic engineering events were registered up to 1999, but none of them were commercialized (Table 2.6). Since 2000, not any new transgenic tomato was registered (http://www.isaaa.org/gmapprovaldatabase/default.asp).

2.6.2 Toolkit for Genetic Engineering Tomato

Tomato genetic transformation was initially established in the 1980s (McCormick et al. 1986). The primary mode of transformation is *Agrobacterium*-mediated procedures by incubating with tomato explants such as leaf, hypocotyl, or cotyledon, followed by the regeneration of plants via shoot organogenesis from callus. Based

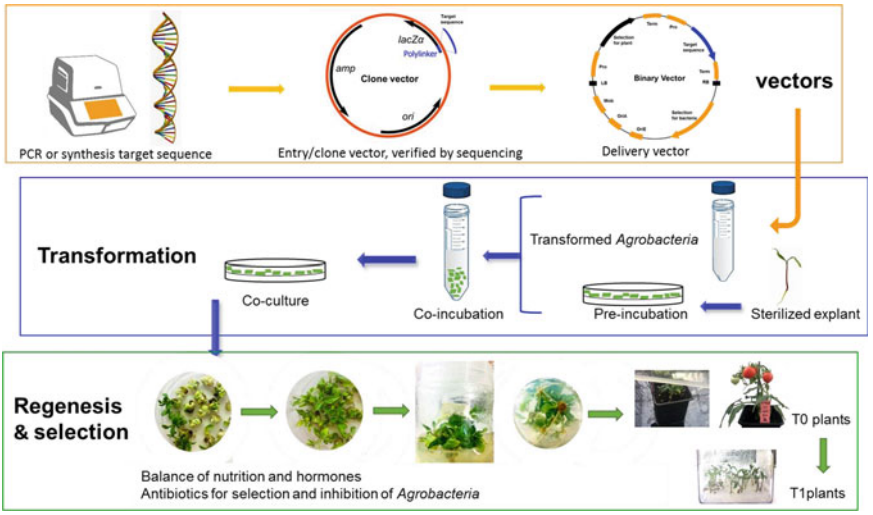

Fig. 2.7 A general workflow for transformation based on widely used protocols. The target sequence could be obtained by PCR or commercial synthesis, and then different cloning methods used to transfer it into the clone vector. After verifying the clone vector, target sequence could be transferred to delivery vector, which is adapted for agrobacteria transformation. Tomato seeds are germinated in sterilized medium. When cotyledons appear, they are cut for pre-culture. After pre-culture, cotyledons (or other explants) are co-incubated with Agrobacteria that carry delivery vector and Ti plasmid, following a short period (such as 2 days) for co-culture. Then explants are transferred to a medium suitable for regeneration and selection. For different steps of regeneration, different nutrition and hormones are needed. When roots appear, transgenic plants are introduced to greenhouse. For T0 plants, the insertion of exogenous modules should be checked. The seeds of T0 plants are planted on medium with selection antibiotic for selecting the transgenic plants

on reported protocols and the review by Bhatia et al. (2004), a general genetic engineering program for tomato requires (Fig. 2.7):

(1) Vectors to deliver engineering modules into agrobacteria and plants;
(2) Integration of the introduced engineering modules into the genome for stable transformation;
(3) In vitro regeneration and selection of transformed plants.

The effective transformation and regeneration are prerequisite steps for utilizing genetic engineering. Transformation efficiency is strongly dependent on the genotype, explant, and plant growth regulators in the medium (reviewed by Gerszberg et al. 2015).

Successful transformation can also be performed either by dipping developing floral buds in the *Agrobacterium* suspension or by injecting *Agrobacterium* into the floral buds. Yasmeen et al. (2009) observed a high transformation frequency, 12–23% for different constructs, while for Sharada et al. (2017), a much lower transformation efficiency (0.25–0.50%) was obtained on floral dips/floral injections. Unlike in

Arabidopsis, for which flower-dipping method became a widely used transformation way (Clough and Bent 1998), in tomato, this methodology has not been efficient.

Gene silencing or expression of heterologous genes in tomato has been used for decades in research. Different from those two conventional genetic engineering methods, genome editing based on CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats) was first proposed on tomato a few years ago (Brooks et al. 2014), but rapidly showed a large potential and wide application for functional gene characterizing, breeding, and domestication.

2.6.2.1 Gene Silencing and Homologous/Heterologous Expression

Gene silencing is usually obtained via antisense (as for FLAVR SAVR), sense, or RNA interfering (RNAi). Scientists have used it to inhibit the unfavorable ripening/softening after tomato harvesting and during a long distance transportation, to remove compounds stimulating allergies (Le et al. 2006), or block seed production resulting in parthenocarpic fruit (Schijlen et al. 2007). Inhibition or better control of fruit ripening and softening is still one of the major challenges for breeders and scientists for commercial perspectives. This purpose was achieved to different degrees by silencing different genes, including those coding pectin methylesterase (Tieman and Handa 1994), expansin protein (Brummell et al. 1999), beta-galactosidase (Smith et al. 2002), ACC synthase (Gupta et al. 2013), transcription factor SlNAC1 (Meng et al. 2016), pectate lyase (Uluisik et al. 2016).

Different from gene silencing strategies which aim to downregulate endogenous genes of tomato, over expression of endogenous or exogenous genes can also be manipulated to study promoters and gene expression, enhance tolerance to biotic/abiotic stresses, and increase the accumulation of secondary metabolites… Promoters (endogenous or exogenous) can be fused with GUS or florescent protein to follow the gene expression pattern. Fernandez et al. (2009) generated novel Gateway destination vectors based on the detailed characterization of series promoters' expression patterns during fruit development and ripening, facilitating tomato genetic engineering. Redox sensitive GFP (roGFP) was also developed to better study the in vivo redox state in tomato (Huang et al. 2014).

Researchers who work on perennial trees such as apple, peach, banana, etc, often used tomato to do heterologous expression of target genes to *in vivo* study the gene function, since the transformation and regeneration techniques are difficult to apply on those species and even when possible, it is time-consuming to pass juvenile phase to obtain fruit phenotypes. In return, the genes from other species, which showed a phenotype on tomato, can be interesting resources for genetic engineering. For instance, apple vacuolar H+ -translocating inorganic pyrophosphatase (MdVHP1) overexpressed in tomato, improved tolerance to salt and drought stress (Dong et al. 2011). Overexpression of banana MYB TF MaMYB3 inhibited starch degradation and delayed fruit ripening (Fan et al. 2018).

Fusing abiotic-driven promoter with functional TF responding to abiotic stress was a promising strategy for improving stress tolerance. Transgenic plants with

the transcription factor CBF driven by ABA-responsive complex (ABTC1) showed enhanced tolerance to chilling, water deficit, and salt stresses without affecting the growth and yield under normal growing conditions (Lee et al. 2003).

The metabolism flux can also be altered to improve fruit qualities, such as volatiles and nutrition compounds. Domínguez et al. (2010) overexpressed genes coding ω-3 fatty acid desaturases, FAD3, and FAD7, resulting in an increase in the 18:3/18:2 ratio in leaves and fruit, and a significant alteration of (Z)-hex-3-enal/hexanal ratio. At MYB12 under the fruit-specific E8, promoter was inserted into tomato genome, activating the genes related to flavonol and hychoxycinnamic ester biosynthesis, leading to accumulation as much as 10% of fruit dry weight (Zhang et al. 2015a, b).

In addition to those remarkable progresses of genetic engineering since 1980s, the most notable progress has been made since the emerging and development of genome-editing tools, such as CRISPR/Cas9.

2.6.2.2 Genome Editing

Unlike genome-editing tools, Zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), which are based on protein–DNA recognition, CRISPR/Cas9 relies on simple RNA–DNA base pairing and the PAM (protospacer adjacent motif) sequence recognition (Gaj et al. 2013). All these tools result in DNA double-strand breaks (DSBs), but CRISPR/Cas9 showed higher efficiency than ZFN and TALEN (Adli 2018). DSB can be repaired either by error-prone non-homology end joining (NHEJ) or homology-directed repair (HDR). Organisms recruit NHEJ or HDR repairing system to induce indel mutations or precise substitution, resulting in knockout or precise-genome editing, respectively. Besides studying the mechanism of CRISPR/Cas9 genome-editing system, scientists also showed enthusiasm for re-engineering CRISPR/Cas9 tools to make them more flexible and increase their fidelity, via making Cas9 nucleases smaller, expanding the targeting scope, and decreasing the off-target rate.

In 2014, the first CRISPR/Cas9 case was reported in tomato (Brooks et al. 2014) and later scientists have explored CRISPR-based engineering on several topics. As CRISPR/Cas9 system can efficiently introduce knockout mutation, it is a useful method to characterize candidate genes from forward genetics or natural mutation. An elegant case of using CRISPR/Cas9 was the production of RIN-knockout mutant, shedding light on an old topic. Tomato *rin* mutants remain firm after harvest and fail to produce red pigmentation and ethylene, thus RIN has long been believed to be indispensable for the induction of ripening. Ito et al. (2017) used CRISPR/Cas9 gene editing to obtain RIN-knockout mutant, which showed moderate red coloring, different from *rin*'s completely fail-to-ripening phenotype. Moreover, using CRISPR/Cas9 to edit *rin* mutant allele partially restored the induction of ripening. Therefore, they showed that RIN is not essential for the initiation of ripening and is a gain-of-function mutation producing a protein actively repressing ripening, rather than a null mutation. This technology has also been used on methylation/demethylation study. A DNA demethylase gene of tomato SlDML2 was mutated by CRISPR/Cas9

to generate loss-of-function mutants, showing a critical role of SlDML2 in tomato fruit ripening possibly via active demethylation of ripening induced genes and the inhibition of ripening-repressed genes (Lang et al. 2017).

Second generation of CRISPR gene-editing tools includes base editing, CRISPR-mediated gene expression regulation, and CRISPR-mediated live cell chromatin imaging (Adli 2018). The probability of gene insertion was increased by the production of landing pad (Danilo et al. 2018) as well as gene knock-in by precise base mutations (Danilo et al. 2019; Veillet et al. 2019). All these strategies are based on manipulation of Cas9, by turning nuclease Cas9 to nickase Cas9 (nCas9) or dead Cas9 (dCas9, catalytically inactive Cas9), but still keeping the capability to recognize specific sequences. The engineered Cas9 can be fused with other enzymes or proteins to enable base editing, gene regulation, or chromatin imaging.

Shimatani et al. (2017) generated marker-free plants with homozygous heritable DNA substitutions by using D10A mutant nCas9At fused with either a human codon-optimized PmCDA1 (nCas9At-PmCDA1Hs) or a version codon-optimized for Arabidopsis (nCas9At-PmCDA1At). It should be mentioned that the offspring of T0 generation also revealed indels, moreover, the rate of substitution was much lower than the rate of indel mutation. It demonstrated the feasibility of base editing for crop improvement even though with a lower rate. Dreissig et al. (2017) showed visualization of telomere repeats in live leaf cells of *Nicotiana benthamiana* by fusing eGFP/mRuby2 to dCas9, and also DNA–protein interactions in vivo via combining CRISPR-dCas9 with fluorescence-labeled proteins. Researchers developed CRISPR interference (CRISPRi) approach with dCas9 binding activity blocking the transcriptional process and thus downregulating gene expressions (Qi et al. 2013).

CRISPR/Cas9 and related second-generation genome-editing tools increase the feasibility and enlarge the applicable scope of biotechnology. With those progresses and the conventional transgenic tools (RNAi, overexpression, and so on), it allows comprehensive breeding to face multiple challenges toward increasing population and climate changes.

2.6.2.3 Comprehensive Genomic Engineering on Tomato

Rodriguez-Leal et al. (2017) focused on three major productivity traits in tomato: fruit size, inflorescence branching, and plant architecture, and used CRISPR/Cas9 to do genome editing of promoters to generate several cis regulatory alleles. They evaluated the phenotypic impact of those variants and provided an efficient approach to select and fix novel alleles controlling the quantitative traits.

Genome editing can also accelerate domestication, as shown by two groups. Li et al. (2018) selected four stress-tolerant wild tomato accessions to introduce desirable traits by using multiplex CRISPR/Cas9 editing. They targeted coding sequences, cis regulatory regions, or upstream open reading frames of genes associated with morphology, flower and fruit production, and ascorbic acid synthesis. The progeny of

edited plants showed domesticated phenotypes yet retained parental disease resistance and salt tolerance. At the same time, Zsögön et al. (2018) chose wild *S. pimpinellifolium* as the starting material to combine agronomically desirable traits with useful wild line traits via editing of six loci that are important for yield and productivity. Engineered tomatoes showed a remarkable increase in fruit size, number, and lycopene content. As the researchers said, those impressive de novo domestication cases pave the way to exploit the genetic diversity present in wild plants.

Genome-editing tools also show big potential for achieving tomato ideotype, for which the concept and design strategies have been explained in Chap. 5. Recently Naves et al. (2019) proposed to engineer tomato to be the biofactory of secondary metabolites, such as capsaicinoids (the metabolites responsible for the burning sensation of hot pepper). Considering that tomato genome presented all the necessary genes for capsaicinoid production, two strategies, transcriptional activator-like effectors (TALEs) or genome engineering for targeted replacement of promoters were suggested to be used in tandem to activate capsaicinoid biosynthesis in the tomato (Naves et al. 2019).

2.6.3 Genetic Engineering for Improving Pest and Pathogen Resistance

A few tomato diseases remain orphan, that is to say, that no natural resistance genes or QTLs have been discovered yet. Moreover, although available from crop wild relatives, breeders may be unable to fully utilize the resistance genes from genetic diversity because of interspecific barriers or because of linkage drag associated to an introgression from a distant species. In that case, resistance might be engineered through biotechnology.

To circumvent the absence of natural resistance, transgenic technologies relying on RNA interference or expression of pathogen-derived sequence have been used to engineer resistance to a number of pathogens. Besides, the ectopic expression of resistance gene could enhance resistance as shown with the introgression of *pvr1*, a recessive gene from *Capsicum chinense*, in tomato that results in dominant broad-spectrum potyvirus resistance (Kang et al. 2007). Nekrasov et al. (2017) also created a transgene-free powdery mildew resistant tomato by genome deletion.

The CRISPR/Cas technology is also expected to accelerate the breeding of cultivars resistant to diseases. Recently, CRISPR/Cas9 system has been used to engineer tomato plants that target the TYLCV genome with Cas9-single guide RNA at the sequences encoding the coat protein (CP) or replicase (Rep) resulting in immunity against TYLCV (Tashkandi et al. 2018). In addition, although still in its infancy, gene editing by CRISPR-nCas9-cytidine deaminase technology might be used to design de novo synthetic functional resistance alleles in tomato, using knowledge about the natural evolution of resistance genes in related species, as demonstrated by Bastet et al. (2019) in *Arabidopsis thaliana*.

2.6.4 Regulatory Status of Gene Edited Plants

Since 2013, CRISPR/Cas9 systems allowed considerable progress in plant genome editing, giving access to cost-effective and efficient transformation compared with previous technologies and making it rapidly accessible to many researchers. However, this emerging method is still developing and scientific efforts continue to be made in order to realize the full potential of the technology. It offers great opportunities, but also creates regulatory challenges. Concerns have been raised over the status of the plants produced by gene editing and classical genetically modified organisms (GMOs) as the technology generates transgene-free plants. Many plant breeders and scientists consider that gene-editing techniques such as CRISPR/Cas9 should be considered as mutagenesis, and thus be exempt from the GMO directive, because they can induce only changes of DNA sequences and not the insertion of foreign genes. But people opposed to GM organisms contend that the deliberate nature of alterations made through gene editing means that they should fall under the GMO directive. In the U.S.A., Canada, and several other countries, CRISPR/Cas induced mutations are exempt from GMO laws and regarded as equivalent to traditional breeding. In Europe, on 25 July 2018, the European Court of Justice (ECJ) ruled that gene edited crops should be subject to the same regulations as conventional GMOs (Callaway 2018). This may have strong consequences on the breeding developments in different countries.

2.7 Conclusion and Prospects

Tomato is a crop widely adapted to very different conditions. Subsequently, it has to respond to many stresses. Molecular markers have permitted the dissection of the genetic bases of complex traits into individual components, the location of many genes/QTLs on chromosomes, which became accessible to selection. Molecular markers have also allowed breeders to access to wild species in a more efficient way than in the past. Exotic libraries, which consist of marker-defined genomic regions taken from wild species and introgressed onto the background of elite crop lines, provide plant breeders with an important opportunity to improve the agricultural performance of modern varieties. Several research consortiums (for genome sequencing, but also for the valorization of genetic resources and traditional varieties) were gathered to study tomato diversity and adaptation.

Since the availability of the reference genome, many new resources (genome sequences, millions of SNPs), tools (databases, methodological tools), and methods (genome editing, crop modeling, and genomic selection) became available and thus breeding should be more efficient.

Better knowledge of physiological processes, metabolic pathways, genes involved as well as the genetic variability of candidate genes, mutant identification, and translational genetics may be used to go further. New growth conditions such as urban horticulture must be taken into account.

It will be important to combine the empirical approach of breeders based on an intimate knowledge of the tomato crop with the power of biotechnologies. Integration of related disciplines will be more and more important to (1) develop more efficient methods to evaluate the impact of environment on the crop, (2) enhance knowledge of the biochemical and molecular bases of the traits, and (3) better understand G x E and to increase the adaptation of new varieties to new conditions.

Some complex questions remain for research: how several stresses interact, how to deal with new pathogens and pests, root x rootstock interaction, reduction of fertilizers. Finally, modeling can help taking into account these aspects and designing new ideotypes optimized to the adverse variable or optimal conditions.

References

Aarts J, Hontelez JGJ, Fischer P, Verkerk R, Vankammen A, Zabel P (1991) Acid phosphatase-11, a tightly linked molecular marker for root-knot nematode resistance in tomato—from protein to gene, using pcr and degenerate primers containing deoxyinosine. Plant Mol Biol 16:647–661

Abraitiene A, Girgzdiene R (2013) Impact of the short-term mild and severe ozone treatments on the potato spindle tuber viroid-infected tomato (*Lycopersicon esculentum* Mill.). Zemdirbyste-Agriculture 100:277–282

Achuo EA, Prinsen E, Hofte M (2006) Influence of drought, salt stress and abscisic acid on the resistance of tomato to *Botrytis cinerea* and *Oidium neolycopersici*. Plant Pathol 55:178–186

Adams SR, Cockshull KE, Cave CRJ (2001) Effect of temperature on the growth and development of tomato fruits. Ann Bot 88:869–877

Adams P, Ho LC (1993) Effects of environment on the uptake and distribution of calcium in tomato and on the incidence of blossom-end rot. Plant Soil 154:127–132

Adato A, Mandel T, Mintz-Oron S, Venger I, Levy D, Yativ M, Domínguez E, Wang Z, De Vos RC, Jetter R, Schreiber L, Heredia A, Rogachev I, Aharoni A (2009) Fruit-surface flavonoid accumulation in tomato is controlled by a SlMYB12-regulated transcriptional network. PLoS Genetics. e1000777. https://doi.org/10.1371/journal.pgen.1000777

Adli M (2018) The CRISPR tool kit for genome editing and beyond. Nat Commun 9(1):1911

Agrama HA, Scott JW (2006) Quantitative trait loci for *tomato yellow leaf curl virus* and *tomato mottle virus* resistance in tomato. J Am Soc Hort Sci 131:267–272

Ahmad A, Zhang Y, Cao X-F (2010) Decoding the epigenetic language of plant development. Mol Plant 3:719–728

Al-Abdallat A, Al-Debei H, Ayad J, Hasan S, Al-Abdallat AM, Al-Debei HS et al (2014) Over-expression of SlSHN1 gene improves drought tolerance by increasing cuticular wax accumulation in tomato. Int J Mol Sci 15:19499–19515

Albacete A, Cantero-Navarro E, Großkinsky DK, Arias CL, Balibrea ME, Bru R, Fragner L, Ghanem ME, González MDLC, Hernández JA et al (2015) Ectopic overexpression of the cell wall invertase gene CIN1 leads to dehydration avoidance in tomato. J Exp Bot 66:863–878

Albacete A, Martínez-Andújar C, Ghanem ME, Acosta M, Sánchez-Bravo J, Asins MJ, et al (2009) Rootstock-mediated changes in xylem ionic and hormonal status are correlated with delayed leaf senescence, and increased leaf area and crop productivity in salinized tomato. Plant Cell Environ 32:928–938

Albert E, Duboscq R, Latreille M, Santoni S, Beukers M, Bouchet JP, Bitton F, Gricourt J, Poncet C, Gautier V et al (2018) Allele-specific expression and genetic determinants of transcriptomic variations in response to mild water deficit in tomato. Plant J 96(3):635–650

Albert E, Gricourt J, Bertin N, Bonnefoi J, Pateyron S, Tamby J-P, Bitton F, Causse M (2016a) Genotype by watering regime interaction in cultivated tomato: lessons from linkage mapping and gene expression. Theor Appl Genet 129:395–418

Albert E, Segura V, Gricourt J, Bonnefoi J, Derivot L, Causse M (2016b) Association mapping reveals the genetic architecture of tomato response to water deficit: focus on major fruit quality traits. J Exp Bot 67:6413–6430

Albrecht E, Escobar M, Chetelat RT (2010) Genetic diversity and population structure in the tomato-like nightshades *Solanum lycopersicoides* and *S. sitiens*. Ann Bot 105:535–554

Alian A, Altman A, Heuer B (2000) Genotypic difference in salinity and water stress tolerance of fresh market tomato cultivars. Plant Sci 152:59–65

Allwood JW, De Vos RCH, Moing A, Deborde C, Erban A, Kopka J, Goodacre R, Hall RD (2011) Plant metabolomics and its potential for systems biology research: background concepts, technology, and methodology. In: Methods Enzymol, 1st edn. https://doi.org/10.1016/b978-0-12-385118-5.00016-5

Almeida J, Quadrana L, Asís R et al (2011) Genetic dissection of vitamin E biosynthesis in tomato. J Exp Bot 62(11):3781–3798

Alpert KB, Tanksley SD (1996) High-resolution mapping and isolation of a yeast artificial chromosome contig containing *fw2.2*: a major fruit weight quantitative trait locus in tomato. Proc Natl Acad Sci USA 93:15503–15507

Alseekh S, Fernie AR (2018) Metabolomics 20 years on: what have we learned and what hurdles remain? Plant J 94:933–942

Alseekh S, Ofner I, Pleban T, Tripodi P, Di Dato F, Cammareri M, Mohammad A, Grandillo S, Fernie AR, Zamir D (2013) Resolution by recombination: breaking up Solanum pennellii introgressions. Trends Plant Sci 18:536–538

Alseekh S, Tong H, Scossa F, Brotman Y, Vigroux F, Tohge T et al (2017) Canalization of tomato fruit metabolism. Plant Cell 29(11):2753–2765

Alseekh S, Tong H, Scossa F, Brotman Y, Vigroux F, Tohge T et al (2017) Canalization of tomato fruit metabolism. Plant Cell 29(11):2753–2765

Ambros V (2004) The functions of animal microRNAs. Nature 431:350–355

Andolfo G, Jupe F, Witek K, Etherington GJ, Ercolano MR, Jones JDG (2014) Defining the full tomato NB-LRR resistance gene repertoire using genomic and cDNA RenSeq. BMC Plant Biol 14

Anfoka G, Moshe A, Fridman L, Amrani L, Rotem O, Kolot M, Zeidan M, Czosnek H, Gorovits R (2016) Tomato yellow leaf curl virus infection mitigates the heat stress response of plants grown at high temperatures. Sci Rep 6:19715

Apse MP, Aharon GS, Snedden WA, Blumwald E (1999) Salt tolerance conferred by overexpression of a vacuolar Na+/H+ antiport in Arabidopsis. Science 285:1256–1258

Arafa RA, Rakha MT, Soliman NEK, Moussa OM, Kamel SM, Shirasawa K (2017) Rapid identification of candidate genes for resistance to tomato late blight disease using next-generation sequencing technologies. PLoS ONE 12:e0189951

Archak S, Karihaloo JL, Jain A (2002) RAPD markers reveal narrowing genetic base of Indian tomato cultivars. Curr Sci 82:1139–1143

Arms EM, Lounsbery JK, Bloom AJ, St. Clair DA (2016) Complex relationships among water use efficiency-related traits, yield, and maturity in tomato lines subjected to deficit irrigation in the field. Crop Sci 56:1698

Ashrafi H, Kinkade MP, Merk HL, Foolad MR (2012) Identification of novel quantitative trait loci for increased lycopene content and other fruit quality traits in a tomato recombinant inbred line population. Mol Breed 30:549–567

Ashrafi-Dehkordi E, Alemzadeh A, Tanaka N, Razi H (2018) Meta-analysis of transcriptomic responses to biotic and abiotic stress in tomato. PeerJ 6:e4631

Asins MJ, Albacete A, Martinez-Andujar C, Pérez-Alfocea F, Dodd IC, Carbonell EA, Dieleman JA (2017) Genetic analysis of rootstock-mediated nitrogen (N) uptake and root-to-shoot signalling at contrasting N availabilities in tomato. Plant Sci 263:94–106

Asins MJ, Bolarín MC, Pérez-Alfocea F, Estañ MT, Martínez-Andújar C, Albacete A et al (2010) Genetic analysis of physiological components of salt tolerance conferred by Solanum rootstocks. What is the rootstock doing for the scion? Theor Appl Genet 121:105–115

Asins MJ, Raga V, Roca D, Belver A, Carbonell EA (2015) Genetic dissection of tomato rootstock effects on scion traits under moderate salinity. Theor Appl Genet 128:667–679

Asins MJ, Villalta I, Aly MM, Olías R, Álvarez De Morales P, Huertas R et al (2013) Two closely linked tomato HKT coding genes are positional candidates for the major tomato QTL involved in Na^+/K^+ homeostasis. Plant Cell Environ 36:1171–1191

Atanassova B (1999) Functional male sterility (ps2) in tomato (*Lycopersicon esculentum* Mill.) and its application in breeding and seed production. Euphytica 107: 1, 13–21

Auerswald H, Schwarz D, Kornelson C, Krumbein A, Brückner B (1999) Sensory analysis, sugar and acid content of tomato at different EC values of the nutrient solution. Sci Hort (Amsterdam) 82:227–242

Bai Y, Lindhout P (2007) Domestication and breeding of tomatoes: what have we gained and what can we gain in the future? Ann Bot 100(5):1085–1094

Bai YL, Huang CC, van der Hulst R, Meijer-Dekens F, Bonnema G, Lindhout P (2003) QTLs for tomato powdery mildew resistance (*Oidium lycopersici*) in *Lycopersicon parviflorum* G1.1601 co-localize with two qualitative powdery mildew resistance genes. Mol Plant-Microbe Interact 16:169–176

Bai YL, Kissoudis C, Yan Z, Visser RGF, van der Linden G (2018) Plant behaviour under combined stress: tomato responses to combined salinity and pathogen stress. Plant J 93:781–793

Bai YL, Pavan S, Zheng Z, Zappel NF, Reinstadler A, Lotti C, De Giovanni C, Ricciardi L, Lindhout P, Visser R, Theres K, Panstruga R (2008) Naturally occurring broad-spectrum powdery mildew resistance in a central American tomato accession is caused by loss of *Mlo* function. Mol Plant-Microbe Interact 21:30–39

Baldazzi V, Bertin N, Jong H, Genard M (2012) Towards multiscale plant models: integrating cellular networks. Trends Plant Sci 17:728–736

Baldazzi V, Génard M, Bertin N (2017) Cell division, endoreduplication and expansion processes: setting the cell and organ control into an integrated model of tomato fruit development. Acta Hort 1182

Baldazzi V, Pinet A, Vercambre G, Benard C, Biais B, Génard M (2013) In-silico analysis of water and carbon relations under stress conditions. A multi-scale perspective centered on fruit. Front Plant Sci 4. https://doi.org/10.3389/fpls.2013.00495

Baldazzi V, Valsesia P, Génard M, Bertin N (2019) Organ-wide and ploidy-dependent regulations both contribute to cell size determination: evidence from a computational model of tomato fruit. J Exp Bot. https://doi.org/10.1093/jxb/erz398

Baldet P, Stevens R, Causse M, Duffe P, Buret M, Rothan C, Garchery C, Duffé P, Carchery C, Baldet P et al (2007) Candidate genes and quantitative trait loci affecting fruit ascorbicacid content in three tomato populations. Plant Physiol 143:1943–1953

Baldwin E, Scott J, Shewmaker C, Schuch W (2000) Flavor trivia and tomato aroma: biochemistry and possible mechanisms for control of important aroma components. HortScience 35:1013–1022

Baldwin EA, Nisperos-Carriedo MO, Baker R, Scott JW (1991) Quantitative analysis of flavor parameters in six Florida tomato cultivars (*Lycopersicon esculentum* Mill). J Agri Food Chem 39:1135–1140

Baldwin EA, Scott JW, Einstein MA, Malundo TMM, Carr BT, Shewfelt RL, Tandon KS (1998) Relationship between sensory and instrumental analysis for tomato flavor. J Am Soc Hort Sci 123:906–915

Ballester A-R, Bovy AG, Viquez-Zamora M, Tikunov Y, Grandillo S, de Vos R, de Maagd RA, van Heusden S, Molthoff J (2016) Identification of loci affecting accumulation of secondary

metabolites in tomato fruit of a *Solanum lycopersicum* × *Solanum chmielewskii* introgression line population. Front Plant Sci 7:1428

Bandillo N, Raghavan C, Muyco P, Sevilla MAL, Lobina IT, Dilla-Ermita C, Tung C-W, McCouch S, Thomson M, Mauleon R et al (2013) Multi-parent advanced generation inter-cross (MAGIC) populations in rice: progress and potential for genetics research and breeding. Rice 6:11

Bastet A, Zafirov D, Giovinazzo N, Guyon-Debast A, Nogué F, Robaglia C, Gallois J-L (2019) Mimicking natural polymorphism in eIF4E by CRISPR-Cas9 base editing is associated with resistance to potyviruses. Plant Biotechnol J. https://doi.org/10.1111/pbi.13096

Bauchet G, Causse M (2012) Genetic diversity in tomato (*Solanum lycopersicum*) and its wild relatives. In: Caliskan M (ed) Genetic Divers Plants. ISBN: 978-953-51-0185-7, InTech, http://www.intechopen.com/books/genetic-diversity-in-plants/genetic-diversity-in-tomatosolanum-lycopersicum-and-its-wild-relatives. https://doi.org/10.5772/33073

Bauchet G, Grenier S, Samson N, Bonnet J, Grivet L, Causse M (2017a) Use of modern tomato breeding germplasm for deciphering the genetic control of agronomical traits by Genome Wide Association study. Theor Appl Genet 130:875–889

Bauchet G, Grenier S, Samson N, Segura V, Kende A, Beekwilder J, Cankar K, Gallois J-L, Gricourt J, Bonnet J et al (2017b) Identification of major loci and genomic regions controlling acid and volatile content in tomato fruit: implications for flavor improvement. New Phytol 215:624–641

Baxter CJ, Liu JL, Fernie AR, Sweetlove LJ (2007) Determination of metabolic fluxes in a non-steady-state system. Phytochemistry 68:2313–2319

Beauvoit B, Belouah I, Bertin N, Belmys Cakpo C, Colombié S, Dai Z, Gautier H, Génard M, Moing A, Roch L, Vercambre G, Gibon Y (2018) Putting primary metabolism into perspective to obtain better fruits. Ann Bot 122(1):1–21

Beauvoit BP, Colombié S, Monier A, Andrieu MH, Biais B, Bérnard C, Chéniclet C, Dieuaide-Noubhani M, Nazaret C, Mazat JP et al (2014) Model-assisted analysis of sugar metabolism throughout tomato fruit development reveals enzyme and carrier properties in relation to vacuole expansion. Plant cell 26(8):3224–3242

Belfanti E, Malatrasi M, Orsi I, Boni AG (2015) Isolated nucleotide sequence from *solanum lycopersicum* for improved resistance to *tomato spotted wilt virus*, TSWV. Patent WO/2015/090468; International Application No: PCT/EP2013/077799

Bernacchi D, Beck-Bunn T, Emmatty D, Eshed Y, Inai S, Lopez J, Petiard V, Sayama H, Uhlig J, Zamir D, Tanksley S (1998) Advanced backcross QTL analysis in tomato. II. Evaluation of near-isogenic lines carrying single-donor introgressions for desirable wild QTL-alleles derived from *Lycopersicon hirsutum* and *L. pimpinellifolium*. Theor Appl Genet 97(1/2): 170–180; erratum 97(7): 1191–1196

Bernacchi D, Beck-Bunn T, Eshed Y, Lopez J, Petiard V, Uhlig J, Zamir D, Tanksley S (1998b) Advanced backcross QTL analysis in tomato. I. Identification of QTLs for traits of agronomic importance from *Lycopersicon hirsutum*. Theor Appl Genet 97:381–397

Berr A, Shafiq S, Shen WH (2011) Histone modifications in transcriptional activation during plant development. Biochim Biophys Acta Gene Regul Mech 1809:567–576

Bertin N, Borel C, Brunel B, Cheniclet C, Causse M (2003) Do genetic make-up and growth manipulation affect tomato fruit size by cell number, or cell size and DNA endoreduplication? Ann Bot 92(3):415–424

Bertin N, Guichard S, Leonardi C, Longuenesse JJ, Langlois D, Navez B (2000) Seasonal evolution of the quality of fresh glasshouse tomatoes under mediterranean conditions, as affected by air vapour pressure deficit and plant fruit load. Ann Bot 85:741–750

Bertin N, Gautier H, Roche C (2002) Number of cells in tomato fruit depending on fruit position and source-sink balance during plant development. Plant Growth Regul 36(2):105–112

Bertin N, Martre P, Génard M, Quilot B, Salon C (2010) Why and how can process-based simulation models link genotype to phenotype for complex traits? Case-study of fruit and grain quality traits. J Exp Bot 61:955–967

Bhatia P, Ashwath N, Senaratna T, Midmore D (2004) Tissue culture studies of tomato (*Lycopersicon esculentum*). Plant Cell Tiss Org Cult 78(1):1–21

Bhatt RM, Srinivasa Rao NK (1987) Seed germination and seedling growth responses of tomato cultivars to imposed water stress. J Hort Sci 62:221–225
Birchler JA, Yao H, Chudalayandi S, Vaiman D, Veitia RA (2010) Heterosis. Plant Cell 22:2105–2112
Blanca J, Cañizares J, Cordero L, Pascual L, Diez MJ, Nuez F (2012) Variation revealed by SNP genotyping and morphology provides insight into the origin of the tomato. PLoS ONE 7:e48198
Blanca J, Montero-Pau J, Sauvage C, Bauchet G, Illa E, Díez MJ, Francis D, Causse M, van der Knaap E, Cañizares J (2015) Genomic variation in tomato, from wild ancestors to contemporary breeding accessions. BMC Genom 16:257
Bloom AJ, Zwieniecki MA, Passioura JB, Randall LB, Holbrook NM, St. Clair DA (2004) Water relations under root chilling in a sensitive and tolerant tomato species. Plant, Cell Environ 27:971–979
Boison SA, Utsunomiya ATH, Santos DJA, Neves HHR, Carvalheiro R, Mészáros G, Utsunomiya YT, do Carmo AS, MA RS, Machado SA et al (2017) Accuracy of genomic predictions in Gyr (Bos indicus) dairy cattle. J Dairy Sci 100:5479–5490
Bolger A, Scossa F, Bolger ME, Lanz C, Maumus F, Tohge T, Quesneville H, Alseekh S, Sørensen I, Lichtenstein G et al (2014) The genome of the stress-tolerant wild tomato species Solanum pennellii. Nat Genet 46:1034–1038
Boote K (2016) Modelling crop growth and yield in tomato cultivation. ID: 9781786760401-010
Boureau L, How-Kit A, Teyssier E, Drevensek S, Rainieri M, Joubès J, Stammitti L, Pribat A, Bowler C, Hong Y et al (2016) A CURLY LEAF homologue controls both vegetative and reproductive development of tomato plants. Plant Mol Biol 90:485–501
Bovy A, de Vos R, Kemper M, Schijlen E, Pertejo MA, Muir S, Collins G, Robinson S, Verhoeyen M, Hughes S, Santos-Buelga C (2002) High-flavonol tomatoes resulting from the heterologous expression of the maize transcription factor genes *LC* and *C1*. Plant Cell 14:2509–2526
Bovy A, Schijlen E, Hall RD (2007) Metabolic engineering of flavonoids in tomato (*Solanum lycopersicum*): the potential for metabolomics. Metabolomics 3:399–412
Boote KJ, Rybak MR, Scholberg JM, Jones JW (2012) Improving the CROPGRO-Tomato model for predicting growth and yield response to temperature. HortScience 47:1038–1049
Brachi B, Morris GP, Borevitz JO (2011) Genome-wide association studies in plants: the missing heritability is in the field. Genome Biol 12:232
Bramley PM (2000) Is lycopene beneficial to human health? Phytochemistry 54:233–236
Brandwagt BF, Mesbah LA, Takken FLW, Laurent PL, Kneppers TJA, Hille J, Nijkamp HJJ (2000) A longevity assurance gene homolog of tomato mediates resistance to Alternaria alternata f. sp lycopersici toxins and fumonisin B(1). In: Proceedings of the national academy of sciences of the United States of America 97:4961-4966
Breiman L (2001) Random forests. Mach Learn 45:5–32
Brommonschenkel SH, Frary A, Tanksley SD (2000) The broad-spectrum tospovirus resistance gene Sw-5 of tomato is a homolog of the root-knot nematode resistance gene Mi. Mol Plant-Microbe Interact 13:1130-1138
Brooks C, Nekrasov V, Lippman ZB, Van Eck J (2014) Efficient gene editing in tomato in the first generation using the clustered regularly interspaced short palindromic repeats/CRISPR-associated9 system. Plant Physiol 166(3):1292–1297
Brouwer DJ, Jones ES, St Clair DA (2004) QTL analysis of quantitative resistance to *Phytophthora infestans* (late blight) in tomato and comparisons with potato. Genome 47:475–492
Brouwer DJ, St Clair DA (2004) Fine mapping of three quantitative trait loci for late blight resistance in tomato using near isogenic lines (NILs) and sub-NILs. Theor Appl Genet 108:628–638
Browning BL, Browning SR (2016) Genotype imputation with millions of reference samples. Amer J Hum Genet 98:116–126
Bruhn CM, Feldman N, Garlitz C, Harwood J, Ivans E, Marshall M, Riley A, Thurber D, Williamson E (1991) Consumer perceptions of quality: apricots, cantaloupes, peaches, pears, strawberries, and tomatoes. J Food Qual 14:187–195

Brummell DA, Harpster MH, Civello PM, Palys JM, Bennett AB, Dunsmuir P (1999) Modification of expansin protein abundance in tomato fruit alters softening and cell wall polymer metabolism during ripening. Plant Cell 11(11):2203–2216

Bucheli P, Voirol E, De La Torre R, López J, Rytz A, Tanksley SD, Pétiard V (1999) Definition of nonvolatile markers for flavor of tomato (*Lycopersicon esculentum* Mill.) as tools in selection and breeding. J Agri Food Chem 47:659–664

Budiman MA, Chang S-B, Lee S, Yang TJ, Zhang H-B, de Jong H, Wing RA (2004) Localization of jointless-2 gene in the centromeric region of tomato chromosome 12 based on high resolution genetic and physical mapping. Theor Appl Genet 108:190–196

Bush DS (1995) Calcium regulation in plant cells and its role in signaling. Annu Rev Plant Physiol 46:95–122

Bussières P (1994) Water import rate in tomato fruit: a resistance model. Ann Bot 73:75–82

Butler L (1952) The linkage map of the tomato. J Hered 43:25–36

Cagas CC, Lee ON, Nemoto K, Sugiyama N (2008) Quantitative trait loci controlling flowering time and related traits in a *Solanum lycopersicum* × *S. pimpinellifolium* cross. Sci Hort (Amsterdam) 116:144–151

Calin GA, Croce CM (2006) MicroRNA signatures in human cancers. Nat Rev Cancer 6:857–866

Callaway E (2018) CRISPR plants now subject to t ough GM l aws in European Union. Nature 560:16. https://doi.org/10.1038/d41586-018-05814-6

Calus MPL, Meuwissen THE, de Roos APW, Veerkamp RF (2008) Accuracy of genomic selection using different methods to define haplotypes. Genetics 178:553–561

Canady MA, Meglic V, Chetelat RT (2005) A library of *Solanum lycopersicoides* introgression lines in cultivated tomato. Genome 48:685–697

Cantero-Navarro E, Romero-Aranda R, Fernández-Muñoz R, Martínez-Andújar C, Pérez-Alfocea F, Albacete A (2016) Improving agronomic water use efficiency in tomato by rootstock-mediated hormonal regulation of leaf biomass. Plant Sci 251:90–100

Cao K, Xu H, Zhang R, Xu D, Yan L, Sun Y, Xia L, Zhao J, Zou Z, Bao E (2019) Renewable and sustainable strategies for improving the thermal environment of Chinese solar greenhouses. Energy Build. In Press

Cárdenas PD, Sonawane PD, Pollier J, Vanden Bossche R, Dewangan V, Weithorn E, Tal L, Meir S, Rogachev I, Malitsky S, Giri AP, Goossens A, Burdman S, Aharoni A (2016) GAME9 regulates the biosynthesis of steroidal alkaloids and upstream isoprenoids in the plant mevalonate pathway. Nat Commun 7:10654

Carelli BP, Gerald LTS, Grazziotin FG, Echeverrigaray S (2006) Genetic diversity among Brazilian cultivars and landraces of tomato *Lycopersicon esculentum* Mill. revealed by RAPD markers. Genet Resour Crop Evol 53:395–400

Carmeille A, Caranta C, Dintinger J, Prior P, Luisetti J, Besse P (2006) Identification of QTLs for *Ralstonia solanacearum* race 3-phylotype II resistance in tomato. Theor Appl Genet 113:110–121

Carmel-Goren L, Liu YS, Lifschitz E, Zamir D (2003) The *SELF-PRUNING* gene family in tomato. Plant Mol Biol 52:1215–1222

Caro M, Cruz V, Cuartero J, Estañ MT, Bolarin MC (1991) Salinity tolerance of normal-fruited and cherry tomato cultivars. Plant Soil 136:249–255

Caromel B, Hamers C, Touhami N, Renaudineau A, Bachellez A, Massire A, Damidaux R, Lefebvre V (2015) Screening tomato germplasm for resistance to late blight. In: INNOHORT, innovation in integrated & organic horticulture. ISHS International Symposium, Avignon, France, 8–12 June 2015, pp 15–16

Carrari F, Baxter C, Usadel B, Urbanczyk-Wochniak E, Zanor M-I, Nunes-Nesi A, Nikiforova V, Centero D, Ratzka A, Pauly M et al (2006) Integrated analysis of metabolite and transcript levels reveals the metabolic shifts that underlie tomato fruit development and highlight regulatory aspects of metabolic network behavior. Plant Physiol 142:1380–1396

Casteel CL, Walling LL, Paine TD (2007) Effect of *Mi-1.2* gene in natal host plants on behavior and biology of the tomato psyllid *Bactericerca cockerelli* (Sulc) (*Hemiptera: Psyllidae*). J Entomol Sci 42:155–162

Catanzariti AM, Do HTT, Bru P, de Sain M, Thatcher LF, Rep M, Jones DA (2017) The tomato I gene for Fusarium wilt resistance encodes an atypical leucine-rich repeat receptor-like protein whose function is nevertheless dependent on SOBIR1 and SERK3/BAK1. Plant J 89:1195–1209

Catanzariti AM, Lim GTT, Jones DA (2015) The tomato I-3 gene: a novel gene for resistance to Fusarium wilt disease. New Phytol 207:106–118

Catchen JM, Boone JQ, Davey JW, Hohenlohe PA, Etter PD, Blaxter ML (2011) Genome-wide genetic marker discovery and genotyping using next-generation sequencing. Nat Rev Genet 12:499–510

Causse M, Buret M, Robini K, Verschave P (2003) Inheritance of nutritional and sensory quality traits in fresh market tomato and relation to consumer preferences. J Food Sci 68:2342–2350

Causse M, Friguet C, Coiret C, Lépicier M, Navez B, Lee M, Holthuysen N, Sinesio F, Moneta E, Grandillo S (2010) Consumer preferences for fresh tomato at the European scale: a common segmentation on taste and firmness. J Food Sci 75(9):531–541

Causse M, Chaïb J, Lecomte L, Buret M, Hospital F (2007a) Both additivity and epistasis control the genetic variation for fruit quality traits in tomato. Theor Appl Genet 115:429–442

Causse M, Duffe P, Gomez MC, Buret M, Damidaux R, Zamir D, Gur A, Chevalier C, Lemaire-Chamley M, Rothan C (2004) A genetic map of candidate genes and QTLs involved in tomato fruit size and composition. J Exp Bot 55:1671–1685

Causse M, Damidaux R, Rousselle P (2007) Traditional and enhanced breeding for fruit quality traits in tomato. In: Razdan MK, Mattoo AK (eds) Genetic improvement of solanaceous crops, Vol. 2: Tomato. Science Publishers, Enfield, USA, pp 153–192

Causse M, Saliba-Colombani V, Lesschaeve I, Buret M (2001) Genetic analysis of organoleptic quality in fresh market tomato. 2. Mapping QTLs for sensory attributes. Theor Appl Genet 102:273–283

Causse M, Saliba-Colombani V, Lecomte L, Duffé P, Rousselle P, Buret M (2002) QTL analysis of fruit quality in fresh market tomato: a few chromosome regions control the variation of sensory and instrumental traits. J Exp Bot 53:2089–2098

Causse M, Desplat N, Pascual L et al (2013) Whole genome resequencing in tomato reveals variation associated with introgression and breeding events. BMC Genomics 14, 791

Chakrabarti M, Zhang N, Sauvage C, Muños S, Blanca J, Cañizares J, Diez MJ, Schneider R, Mazourek M, McClead J et al (2013) A cytochrome P450 regulates a domestication trait in cultivated tomato. Proc Natl Acad Sci USA 110:17125–17130

Chen FQ, Foolad MR, Hyman J, St. Clair DA, Beelaman RB (1999) Mapping of QTLs for lycopene and other fruit traits in a *Lycopersicon esculentum* × *L. pimpinellifolium* cross and comparison of QTLs across tomato species. Mol Breed 5:283–299

Chen J, Kang S, Du T, Qiu R, Guo P, Chen R (2013) Quantitative response of greenhouse tomato yield and quality to water deficit at different growth stages. Agri Water Manag 129:152–162

Chen X (2005) microRNA biogenesis and function in plants. FEBS Lett 579:5923

Chen X (2009) Small RNAs and their roles in plant development. Annu Rev Cell Dev Biol 25:21–44

Chetelat RT, DeVerna JW, Bennett AB (1995) Introgression into tomato (*Lycopersicon esculentum*) of the *L. chmielewski*i sucrose accumulator gene (sucr) controlling fruit sugar composition. Theor Appl Genet 91:327–333

Cho SK, Ben Chaabane S, Shah P, Poulsen CP, Yang SW (2014) COP1 E3 ligase protects HYL1 to retain microRNA biogenesis. Nat Commun 5:5867

Chunwongse J, Chunwongse C, Black L, Hanson P (2002) Molecular mapping of the *Ph-3* gene for late blight resistance in tomato. J Hort Sci Biotechnol 77:281–286

Clark AG (2004) The role of haplotypes in candidate gene studies. Genet Epidemiol 27:321–333

Clough SJ, Bent AF (1998) Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J16(6):735–743

Coaker GL, Francis DM (2004) Mapping, genetic effects, and epistatic interaction of two bacterial canker resistance QTLs from *Lycopersicon hirsutum*. Theor Appl Genet 108:1047–1055

Colliver S, Bovy A, Collins G, Muir S, Robinson S, de Vos CHR, Verhoeyen ME (2002) Improving the nutritional content of tomatoes through reprogramming their flavonoid biosynthetic pathway. Phytochem Rev 1:113–123

Colombié S, Beauvoit B, Nazaret C, Bénard C, Vercambre G, Le Gall S, Biais B, Cabasson C, Maucourt M, Bernillon S, Moing A, Dieuaide-Noubhani M, Mazat J-P, Gibon Y (2017) Respiration climacteric in tomato fruits elucidated by constraint-based modelling. New Phytol 213:1726–1739

Colombié S, Nazaret C, Bénard C, Biais B, Mengin V, Solé M, Fouillen L, Dieuaide-Noubhani M, Mazat J-P, Beauvoit B, Gibon Y (2015) Modelling central metabolic fluxes by constraint-based optimization reveals metabolic reprogramming of developing *Solanum lycopersicum* (tomato) fruit. Plant J 81:24–39

Comai L, Henikoff S (2006) TILLING: practical single-nucleotide mutation discovery. Plant J 45:684–694

Coneva V, Frank MH, Balaguer MAL, Li M, Sozzani R, Chitwood DH (2017) Genetic architecture and molecular networks underlying leaf thickness in desert-adapted Tomato *Solanum pennellii*. Plant Physiol 175(1):376–391

Constantinescu D, Memmah M-M, Vercambre G, Génard M, Baldazzi V, Causse M et al (2016) Model-assisted estimation of the genetic variability in physiological parameters related to tomato fruit growth under contrasted water conditions. Front Plant Sci 7:1841. https://doi.org/10.3389/fpls.2016.01841

Costa JM, Ortuño MF, Chaves MM (2007) Deficit irrigation as a strategy to save water: physiology and potential application to horticulture. J Integr Plant Biol 49:1421–1434

Cournède P-H et al (2013) Development and evaluation of plant growth models: methodology and implementation in the pygmalion platform. Math Mod Nat Phen 8(4):112–130

Cowger C, Brown JKM (2019) Durability of quantitative resistance in crops: greater than we know? Annu Rev Phytopathol 57:253–277

Crain J, Mondal S, Rutkoski J, Singh RP, Poland J (2018) Combining high-Throughput phenotyping and genomic information to increase prediction and selection accuracy in wheat breeding. Plant Genome 11: 0

Crossa J, Pérez-Rodríguez P, Cuevas J, Montesinos-López O, Jarquín D, de los Campos G, Burgueño J, Camacho-González JM, Pérez-Elizalde S, Beyene Y, et al (2017) Genomic selection in plant breeding: methods, models, and perspectives. Trends Plant Sci. https://doi.org/10.1016/j.tplants.2017.08.011

Cui J, Jiang N, Zhou X, Hou X, Yang G, Meng J, Luan Y (2018) Tomato MYB49 enhances resistance to Phytophthora infestans and tolerance to water deficit and salt stress. Planta 248:1487–1503

Cui J, You C, Chen X (2017a) The evolution of microRNAs in plants. Curr Opin Plant Biol 35:61–67

Cui J, Zhou B, Ross SA, Zempleni J (2017b) Nutrition, microRNAs, and human health. Adv Nutr 8:105–112

Cuyabano BC, Su G, Lund MS (2014) Genomic prediction of genetic merit using LD-based haplotypes in the Nordic Holstein population. BMC Genom. https://doi.org/10.1186/1471-2164-15-1171

Cuyabano BCD, Su G, Lund MS (2015a) Selection of haplotype variables from a high-density marker map for genomic prediction. Genet Sel Evol 47:61

Cuyabano BCD, Su G, Rosa GJM, Lund MS, Gianola D (2015b) Bootstrap study of genome-enabled prediction reliabilities using haplotype blocks across Nordic Red cattle breeds. J Dairy Sci 98:7351–7363

Dal Cin V, Kevany B, Fei Z, Klee HJ (2009) Identification of Solanum habrochaites loci that quantitatively influence tomato fruit ripening-associated ethylene emissions. Theor Appl Genet 119:1183–1192

Danecek P, Huang J, Min JL, Timpson NJ, Trabetti E, Richards JB, Durbin R, Howie B, Gambaro G, Zheng H-F et al (2015) Improved imputation of low-frequency and rare variants using the UK10K haplotype reference panel. Nat Commun 6:8111

Danilo B, Perrot L, Botton E, Nogué F, Mazier M (2018) The DFR locus: a smart landing pad for targeted transgene insertion in tomato. PLoS ONE 13(12):e0208395

Danilo B, Perrot L, Mara K, Botton E, Nogué F, Mazier M (2019) Efficient and transgene-free gene targeting using Agrobacterium-mediated delivery of the CRISPR/Cas9 system in tomato. Plant Cell Rep 38(4):459–462

Das S, Forer L, Schönherr S, Sidore C, Locke AE, Kwong A, Vrieze SI, Chew EY, Levy S, McGue M et al (2016) Next-generation genotype imputation service and methods. Nat Genet 48:1284–1287

Davies JN, Hobson GE (1981) The constituents of tomato fruit—the influence of environment, nutrition, and genotype. Crit Rev Food Sci Nutr 15:205–280

Davila Olivas NH, Kruijer W, Gort G, Wijnen CL, van Loon JJA, Dicke M (2017) Genome-wide association analysis reveals distinct genetic architectures for single and combined stress responses in *Arabidopsis thaliana*. New Phytol 213:838–851

Davis J, Yu DZ, Evans W, Gokirmak T, Chetelat RT, Stotz HU (2009) Mapping of loci from *Solanum lycopersicoides* conferring resistance or susceptibility to *Botrytis cinerea* in tomato. Theor Appl Genet 119:305–314

de Freitas ST, Martinelli F, Feng B, Reitz NF, Mitcham EJ (2018) Transcriptome approach to understand the potential mechanisms inhibiting or triggering blossom-end rot development in tomato fruit in response to plant growth regulators. J Plant Growth Regul 37:183–198

de Groot CC, Marcelis LFM, van den Boogaard R, Lambers H (2004) Response of growth of tomato to phosphorus and nitrogen nutrition. Acta Hort 357–364

de Jong CF, Takken FLW, Cai XH, de Wit P, Joosten M (2002) Attenuation of *Cf*-mediated defense responses at elevated temperatures correlates with a decrease in elicitor-binding sites. Mol Plant-Microbe Interact 15:1040–1049

de Los Campos G, Hickey JM, Pong-Wong R, Daetwyler HD, Calus MPL, Kirst M, Huber D, Peter GF (2013) Whole-genome regression and prediction methods applied to plant and animal breeding. Genetics 193:327–345

De Swaef T, Mellisho CD, Baert A, De Schepper V, Torrecillas A, Conejero W, Steppe K (2014) Model-assisted evaluation of crop load effects on stem diameter variations and fruit growth in peach. Trees 28:1607–1622

Delhaize E, Gruber BD, Ryan PR (2007) The roles of organic anion permeases in aluminium resistance and mineral nutrition. FEBS Lett 581:2255–2262

DeVicente MC, Tanksley SD (1993) QTL analysis of transgressive segregation in an interspecific tomato cross. Genetics 134:585–596

Dileo MV, Pye MF, Roubtsova TV, Duniway JM, MacDonald JD, Rizzo DM, Bostock RM (2010) Abscisic acid in salt stress predisposition to *Phytophthora* root and crown rot in tomato and chrysanthemum. Phytopathology 100:871–879

Diouf IA, Derivot L, Bitton F, Pascual L, Causse M (2018) Water deficit and salinity stress reveal many specific QTL for plant growth and fruit quality traits in tomato. Front Plant Sci 9:279

Dixon MS, Hatzixanthis K, Jones DA, Harrison K, Jones JDG (1998) The tomato *Cf-5* disease resistance gene and six homologs show pronounced allelic variation in leucine-rich repeat copy number. Plant Cell 10:1915–1925

Dixon MS, Jones DA, Keddie JS, Thomas CM, Harrison K, Jones JD (1996) The tomato Cf-2 disease resistance locus comprises two functional genes encoding leucine-rich repeat proteins. Cell 84:451–459

Do PT, Prudent M, Sulpice R, Causse M, Fernie AR (2010) The influence of fruit load on the tomato pericarp metabolome in a Solanum chmielewskii introgression line population. Plant Physiol 154:1128–1142

Doganlar S, Dodson J, Gabor B, Beck-Bunn T, Crossman C, Tanksley SD (1998) Molecular mapping of the *py-1* gene for resistance to corky root rot (*Pyrenochaeta lycopersici*) in tomato. Theor Appl Genet 97:784–788

Doganlar S, Frary A, Ku H-M, Tanksley SD (2003) Mapping quantitative trait loci in inbred backcross lines of *Lycopersicon pimpinellifolium* (LA1589). Genome 45:1189–1202

Domínguez T, Hernández ML, Pennycooke JC, Jiménez P, Martínez-Rivas JM, Sanz C, Stockinger EJ, Sánchez-Serrano JJ, Sanmartín M (2010) Increasing ω-3 desaturase expression in tomato

results in altered aroma profile and enhanced resistance to cold stress. Plant Physiol 153(2):655–665
Donald 1968 C.M. The breeding of crop idéotypes. Euphytica, 17 (1968), pp. 385–403
Dong QL, Liu DD, An XH, Hu DG, Yao YX, Hao YJ (2011) MdVHP1 encodes an apple vacuolar H+-PPase and enhances stress tolerance in transgenic apple callus and tomato. JPlant Physiol 168(17):2124–2133
Dong Z, Men Y, Li Z, Zou Q, Ji J (2019) Chlorophyll fluorescence imaging as a tool for analyzing the effects of chilling injury on tomato seedlings. Sci Hort (Amsterdam) 246:490–497
Dorais M, Papadopoulos AP, Gosselin A (2001) Greenhouse tomato fruit quality. Hortic Rev 26:239–319
Dreissig S, Schiml S, Schindele P, Weiss O, Rutten T, Schubert V, Gladilin E, Mette MF, Puchta H, Houben A (2017) Live-cell CRISPR imaging in plants reveals dynamic telomere movements. Plant J 91(4):565–573
Driedonks N, Wolters-Arts M, Huber H, de Boer G-J, Vriezen W, Mariani C, Rieu I (2018) Exploring the natural variation for reproductive thermotolerance in wild tomato species. Euphytica 214:67
Du Y-D, Niu W-Q, Gu X-B, Zhang Q, Cui B-J (2018) Water- and nitrogen-saving potentials in tomato production: a meta-analysis. Agri Water Manag 210:296–303
Duangjit J, Causse M, Sauvage C (2016) Efficiency of genomic selection for tomato fruit quality. Mol Breed 36(36):29
Edwards SM, Buntjer JB, Jackson R, Bentley AR, Lage J, Byrne E, Burt C, Jack P, Berry S, Flatman E et al (2019) The effects of training population design on genomic prediction accuracy in wheat. Theor Appl Genet 443267
El-hady E, Haiba A, El-hamid NRA, Rizkalla A, Phylogenetic AR (2010) Phylogenetic diversity and relationships of some tomato varieties by electrophoretic protein and RAPD analysis. J Amer Sci 6:434–441
Elvanidi A, Katsoulas N, Augoustaki D, Loulou I, Kittas C (2018) Crop reflectance measurements for nitrogen deficiency detection in a soilless tomato crop. Biosyst Eng 176:1–11
Endelman JB (2011) Ridge regression and other kernels for genomic selection with R package rrBLUP. Plant Genome J 4:250
Ercolano MR, Sanseverino W, Carli P, Ferriello F, Frusciante L (2012) Genetic and genomic approaches for R-gene mediated disease resistance in tomato: retrospects and prospects. Plant Cell Rep 31:973–985
Eriksson EM, Bovy A, Manning K, Harrison L, Andrews J, De Silva J, Tucker GA, Seymour GB, Thompson J, Tor M et al (2004) Effect of the colorless non-ripening mutation on cell wall biochemistry and gene expression during tomato fruit development and ripening 1[w]. Plant Physiol 136:4184–4197
Ernst K, Kumar A, Kriseleit D, Kloos DU, Phillips MS, Ganal MW (2002) The broad-spectrum potato cyst nematode resistance gene (Hero) from tomato is the only member of a large gene family of NBS-LRR genes with an unusual amino acid repeat in the LRR region. Plant J 31:127–136
Eshed Y, Gera G, Zamir D (1996) A genome-wide search for wild-species alleles that increase horticultural yield of processing tomato. Theor Appl Genet 93:877–886
Eshed Y, Zamir D (1995) An introgression line population of *Lycopersicon pennellii* in the cultivatedtomato enables the identification and fine mapping of yield-associated QTL. Genetics 141:1147–1162
Estañ MT, Villalta I, Bolarín MC, Carbonell EA, Asins MJ (2009) Identification of fruit yield loci controlling the salt tolerance conferred by solanum rootstocks. Theor Appl Genet 118:305–312
Evangelou E, Ioannidis JPA (2013) Meta-analysis methods for genome-wide association studies and beyond. Nat Rev Genet 14:379–389
Fan ZQ, Ba LJ, Shan W, Xiao YY, Lu WJ, Kuang JF, Chen JY (2018) A banana R2R3-MYB transcription factor MaMYB3 is involved in fruit ripening through modulation of starch degradation by repressing starch degradation-related genes and MabHLH6. Plant J 96(6):1191–1205
Fang X, Cui Y, Li Y, Qi Y (2015) Transcription and processing of primary microRNAs are coupled by Elongator complex in Arabidopsis. Nat Plants 1:15075

Fanwoua J, de Visser PHB, Heuvelink E, Yin X, Struik PC, Marcelis LFM (2013) A dynamic model of tomato fruit growth integrating cell division, cell growth and endoreduplication. Funct Plant Biol 40(11):1098–1114

FAO (2015) Coping with climate change—the roles of genetic resources for food and agriculture

Farashi S, Kryza T, Clements J, Batra J (2019) Post-GWAS in prostate cancer: from genetic association to biological contribution. Nat Rev Cancer 19:46–59

Fereres E, Soriano MA (2006) Deficit irrigation for reducing agricultural water use. J Exp Bot 58:147–159

Fernandes SB, Dias KOG, Ferreira DF, Brown PJ (2018) Efficiency of multi-trait, indirect, and trait-assisted genomic selection for improvement of biomass sorghum. Theor Appl Genet 131:747–755

Fernandez AI, Viron N, Alhagdow M, Karimi M, Jones M, Amsellem Z, Sicard A, Czerednik A, Angenent G, Grierson D, May S (2009) Flexible tools for gene expression and silencing in tomato. Plant Physiol 151(4):1729–1740

Fernie AR, Aharoni A, Willmitzer L, Stitt M, Tohge T, Kopka J, Carroll AJ, Saito K, Fraser PD, DeLuca V (2011) Recommendations for reporting metabolite data. Plant Cell 23:2477–2482

Fernie AR, Schauer N (2009) Metabolomics-assisted breeding: a viable option for crop improvement? Trends Genet 25:39–48

Finkers R, Bai YL, van den Berg P, van Berloo R, Meijer-Dekens F, ten Have A, van Kan J, Lindhout P, van Heusden AW (2008) Quantitative resistance to *Botrytis cinerea* from *Solanum neorickii*. Euphytica 159:83–92

Finkers R, van den Berg P, van Berloo R, ten Have A, van Heusden AW, van Kan JAL, Lindhout P (2007a) Three QTLs for *Botrytis cinerea* resistance in tomato. Theor Appl Genet 114:585–593

Finkers R, Van Heusden AW, Meijer-Dekens F, Van Kan JAL, Maris P, Lindhout P (2007b) The construction of a Solanum habrochaites LYC4 introgression line population and the identification of QTLs for resistance to Botrytis cinerea. Theor Appl Genet 114:1071–1080

Fishman S, Génard M (1998) A biophysical model of fruit growth: simulation of seasonal and diurnal dynamics of mass. Plant Cell Environ 21:739–752

Foolad MR (2007) Genome mapping and molecular breeding of tomato. Int J Plant Genomics 2007:64358

Foolad MR, Merk HL, Ashrafi H (2008) Genetics, genomics and breeding of late blight and early blight resistance in tomato. Crit Rev Plant Sci 27:75–107

Foolad MR, Panthee DR (2012) Marker-assisted selection in tomato breeding. Crit Rev Plant Sci 31:93–123

Foolad MR, Sullenberger MT, Ohlson EW, Gugino BK (2014) Response of accessions within tomato wild species, Solanum pimpinellifolium to late blight. Plant Breed 133:401–411

Foolad MR, Zhang LP, Khan AA, Nino-Liu D, Lin GY (2002) Identification of QTLs for early blight (*Alternaria solani*) resistance in tomato using backcross populations of a *Lycopersicon esculentum* x *L. hirsutum* cross. Theor Appl Genet 104:945–958

Fragkostefanakis S, Mesihovic A, Simm S, Paupière MJ, Hu Y, Paul P, Mishra SK, Tschiersch B, Theres K, Bovy A et al (2016) HsfA2 controls the activity of developmentally and stress-regulated heat stress protection mechanisms in tomato male reproductive tissues. Plant Physiol 170:2461–2477

Fragkostefanakis S, Röth S, Schleiff E, Scharf KD (2015) Prospects of engineering thermotolerance in crops through modulation of heat stress transcription factor and heat shock protein networks. Plant, Cell Environ 38:1881–1895

Frary A, Doganlar S, Daunay MC, Tanksley SD (2003) QTL analysis of morphological traits in eggplant and implications for conservation of gene function during evolution of solanaceous species. Theor Appl Genet 107:359–370

Frary A, Fulton TM, Zamir D, Tanksley SD (2004) Advanced backcross QTL analysis of a *Lycopersicon esculentum* × *L. pennellii* cross and identification of possible orthologs in the Solanaceae. Theor Appl Genet 108:485–496

Frary A, Keleş D, Pinar H, Göl D, Doğanlar S (2011) NaCl tolerance in *Lycopersicon pennellii* introgression lines: QTL related to physiological responses. Biol Plant 55:461–468

Frary A, Nesbitt TC, Frary A, Grandillo S, Van Der Knaap E, Cong B, Liu J, Meller J, Elber R, Alpert KB et al (2000) fw2.2: a quantitative trait locus key to the evolution of tomato fruit size. Science (80-) 289: 85–88

Frary A, Göl D, Keleş D, Ökmen B, Pınar H, Şığva HÖ et al (2010) Salt tolerance in Solanum pennellii: antioxidant response and related QTL. BMC Plant Biol 10:58

Fridman E, Carrari F, Liu YS, Fernie AR, Zamir D (2004) Zooming in on a quantitative trait for tomato yield using interspecific introgressions. Science (80-) 305: 1786–1789

Fridman E, Liu YS, Carmel-Goren L, Gur A, Shoresh M, Pleban T, Eshed Y, Zamir D (2002) Two tightly linked QTLs modify tomato sugar content via different physiological pathways. Mol Genet Genom 266: 821–826

Fridman E, Pleban T, Zamir D (2000) A recombination hotspot delimits a wild-species quantitative trait locus for tomato sugar content to 484 bp within an invertase gene. Proc Natl Acad Sci USA 97:4718–4723

Fridman E, Zamir D (2003) Functional divergence of a syntenic invertase gene family in tomato, potato, and Arabidopsis. Plant Physiol 131:603–609

Fry WE, Goodwin SB (1997) Re-emergence of potato and tomato late blight in the United States. Plant Dis 81:1349–1357

Fujita M, Fujita Y, Noutoshi Y, Takahashi F, Narusaka Y, Yamaguchi-Shinozaki K, Shinozaki K (2006) Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. Curr Opin Plant Biol 9:436–442

Fulop D, Ranjan A, Ofner I, Covington MF, Chitwood DH, West D, Ichihashi Y, Headland L, Zamir D, Maloof JN, et al. (2016) A new advanced backcross tomato population enables high resolution leaf QTL mapping and gene identification. G3: GenesGenomesGenet 6:3169–3184

Fulton TM (2002) Identification, analysis, and utilization of conserved ortholog set markers for comparative genomics in higher plants. Plant Cell 14:1457–1467

Fulton TM, Beck-Bunn T, Emmatty D, Eshed Y, Lopez J, Petiard V, Uhlig J, Zamir D, Tanksley SD (1997) QTL analysis of an advanced backcross of *Lycopersicon peruvianum* to the cultivated tomato and comparisons with QTLs found in other wild species. Theor Appl Genet 95:881–894

Fulton TM, Grandillo S, Beck-Bunn T, Fridman E, Frampton A, Lopez J, Petiard V, Uhlig J, Zamir D, Tanksley SD (2000) Advanced backcross QTL analysis of a *Lycopersicon esculentum* × *Lycopersicon parviflorum* cross. Theor Appl Genet 100:1025–1042

Gaion LA, Muniz JC, Barreto RF, D'Amico-Damião V, de Mello Prado R, Carvalho RF (2019) Amplification of gibberellins response in tomato modulates calcium metabolism and blossom end rot occurrence. Sci Hortic (Amsterdam) 246:498–505

Gaj T, Gersbach CA, Barbas CF (2013) ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol 31(7):397–405

Gallusci P, Hodgman C, Teyssier E, Seymour GB (2016) DNA methylation and chromatin regulation during fleshy fruit development and ripening. Front Plant Sci 7:807

Gao C, Ju Z, Cao D, Zhai B, Qin G, Zhu H, Fu D, Luo Y, Zhu B (2015) MicroRNA profiling analysis throughout tomato fruit development and ripening reveals potential regulatory role of RIN on microRNAs accumulation. Plant Biotechnol J 13:370–382

Gao L, Gonda I, Sun H et al (2019) The tomato pan-genome uncovers new genes and a rare allele regulating fruit flavor. Nat Genet 51:1044–1051

Garcia V, Bres C, Just D, Fernandez L, Tai FWJ, Mauxion JP, Le Paslier MC, Bérard A, Brunel D, Aoki K et al (2016) Rapid identification of causal mutations in tomato EMS populations via mapping-by-sequencing. Nat Protoc 11:2401–2418

Gauffier C, Lebaron C, Moretti A, Constant C, Moquet F, Bonnet G, Caranta C, Gallois J-L (2016) A TILLING approach to generate broad-spectrum resistance to potyviruses in tomato is hampered by *eIF4E* gene redundancy. Plant J 85:717–729

Gautier H, Diakou-Verdin V, Bénard C, Reich M, Buret M, Bourgaud F, Poëssel JL, Caris-Veyrat C, Génard M (2008) How does tomato quality (sugar, acid, and nutritional quality) vary with ripening stage, temperature, and irradiance? J Agri Food Chem 56:1241–1250

Génard M, Bertin N, Gautier H, Lescourret F, Quilot B (2010) Virtual profiling: a new way to analyse phenotypes. Plant J 62:344–355
Génard M, Lescourret F (2004) Modelling fruit quality: ecophysiological, agronomical and ecological perspectives. In: Dris R, Jain SM (eds) Production practices and quality assessment of food crops, vol 1. Preharvest practice. Kluwer Academic Publisher, Netherlands, pp 47–82
Génard M, Memmah M-M, Quilot-Turion B, Vercambre G, Baldazzi V, Le Bot J, Bertin N, Gautier H, Lescourret F, Pagès L (2016) Process-based simulation models are essential tools for virtual profiling and design of ideotypes: example of fruit and root. In: Yin X, Struik PC (eds) Crop systems biology: narrowing the gaps between crop modelling and genetics, pp 83–104
Gerszberg A, Hnatuszko-Konka K, Kowalczyk T, Kononowicz AK (2015) Tomato (*Solanum lycopersicum* L.) in the service of biotechnology. Plant Cell Tiss Org Cult 120(3):881–902
Geshnizjani N, Ghaderi-Far F, Willems LAJ, Hilhorst HWM, Ligterink W (2018) Characterization of and genetic variation for tomato seed thermo-inhibition and thermo-dormancy. BMC Plant Biol 18:229
Gest N, Gautier H, Stevens R (2013) Ascorbate as seen through plant evolution: the rise of a successful molecule? J Exp Bot 64:33–53
Gianola D, van Kaam JBCHM (2008) Reproducing Kernel Hilbert spaces regression methods for genomic assisted prediction of quantitative traits. Genetics 178:2289
Giovannoni J, Nguyen C, Ampofo B, Zhong S, Fei Z (2017) The epigenome and transcriptional dynamics of fruit ripening. Annu Rev Plant Biol 68:61–84
Giovannucci E (1999) Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiologic literature. J Natl Cancer Inst 91:317–331
Giroux RW, Filion WG (1992) A comparison of the chilling-stress response in two differentially tolerant cultivars of tomato (*Lycopersicon esculentum*). Biochem Cell Biol 70:191–198
Goff SA, Klee HJ (2006) Plant volatile compounds: sensory cues for health and nutritional value? Science 311:815–819
Gonatopoulos-Pournatzis T, Cowling VH (2015) Cap-binding complex (CBC). Biochem J 458:185
Gonzalez-Cendales Y, Catanzariti AM, Baker B, McGrath DJ, Jones DA (2016) Identification of I-7 expands the repertoire of genes for resistance to Fusarium wilt in tomato to three resistance gene classes. Mol Plant Pathol 17:448–463
Goodwin S, McPherson JD, McCombie WR (2016) Coming of age: ten years of next-generation sequencing technologies. Nat Rev Genet 17:333–351
Grandillo S, Cammareri M (2016) Molecular mapping of quantitative trait loci in tomato. In: Causse M, Giovannoni J, Bouzayen M, Zouine M (eds) The tomato genome. Springer, Berlin, pp 39–73
Grandillo S, Chetelat R, Knapp S, Spooner D, Peralta I, Cammareri M, Perez O, Termolino P, Tripodi P, Chiusano ML et al (2011) Solanum sect. Lycopersicon. In: Kole C (ed) Wild crop relatives: genomic and breeding resources. Springer, Berlin, pp 129–215
Grandillo S, Ku HM, Tanksley SD (1996) Characterization of fs8.1, a major QTL influencing fruit shape in tomato. Mol Breed 2:251–260
Grandillo S, Ku HM, Tanksley SD (1999) Identifying the loci responsible for natural variation in fruit size and shape in tomato. Theor Appl Genet 99:978–987
Grandillo S, Tanksley SD (1996a) Genetic analysis of RFLPs, GATA microsatellites and RAPDs in a cross between *L. esculentum* and *L. pimpinellifolium*. Theor Appl Genet 92:957–965
Grandillo S, Tanksley SD (1996b) QTL analysis of horticultural traits differentiating the cultivated tomato from the closely related species *Lycopersicon pimpinellifolium*. Theor Appl Genet 92:935–951
Grandillo S, Termolino P, van der Knaap E (2013) Molecular mapping of complex traits in tomato. In: Liedl BE, Labate JA, Stommel JR, Slade A, Kole C (eds) Genetics, genomics and breeding of tomato. CRC Press, Boca Raton, FL, pp 150–227
Grierson D (2016) Identifying and silencing tomato ripening genes with antisense genes. Plant Biotechnol J14(3):835–838
Grilli G, Trevizan Braz L, Gertrudes E, Lemos M (2007) QTL identification for tolerance to fruit set in tomatoby fAFLP markers. Crop Breed Appl Biotechnol 7:234–241

Grimson A, Srivastava M, Fahey B, Woodcroft BJ, Chiang HR, King N, Degnan BM, Rokhsar DS, Bartel DP (2008) Early origins and evolution of microRNAs and Piwi-interacting RNAs in animals. Nature 455:1193–1197

Guan Y, Stephens M (2008) Practical issues in imputation-based association mapping. PLoS Genet. https://doi.org/10.1371/journal.pgen.1000279

Guichard S, Bertin N, Leonardi C, Gary C (2001) Tomato fruit quality in relation to water and carbon fluxes. Agronomie 21:385–392

Guichard S, Gary C, Leonardi C, Bertin N (2005) Analysis of growth and water relations of tomato fruits in relation to air vapor pressure deficit and plant fruit load. J Plant Growth Regul 24:201–213

Gupta A, Pal RK, Rajam MV (2013) Delayed ripening and improved fruit processing quality in tomato by RNAi-mediated silencing of three homologs of 1-aminopropane-1-carboxylate synthase gene. JPlant Physiol 170(11):987–995

Gur A, Osorio S, Fridman E, Fernie AR, Zamir D (2010) hi2-1, A QTL which improves harvest index, earliness and alters metabolite accumulation of processing tomatoes. Theor Appl Genet 121:1587–1599

Gur A, Semel Y, Osorio S, Friedmann M, Seekh S, Ghareeb B et al (2011) Yield quantitative trait loci from wild tomato are predominately expressed by the shoot. Theor Appl Genet 122:405–420

Gur A, Zamir D (2015) Mendelizing all components of a pyramid of three yield QTL in tomato. Front Plant Sci 6:1096. https://doi.org/10.3389/fpls.2015.01096

Haanstra JPW, Wye C, Verbakel H, Meijer-Dekens F, Van Den Berg P, Odinot P, Van Heusden AW, Tanksley S, Lindhout P, Peleman J (1999) An integrated high-density RFLP-AFLP map of tomato based on two *Lycopersicon esculentum x L. pennellii* F2 populations. Theor Appl Genet 99:254–271

Habier D, Fernando RL, Kizilkaya K, Garrick DJ (2011) Extension of the bayesian alphabet for genomic selection. BMC Bioinformatics 12:186

Hagassou D, Francia E, Ronga D, Buti M (2019) Blossom end-rot in tomato (Solanum lycopersicum L.): A multi-disciplinary overview of inducing factors and control strategies. Sci Hortic (Amsterdam) 249:49–58

Haggard JE, Johnson EB, St. Clair DA (2013) Linkage relationships among multiple QTL for horticultural traits and late blight (P. infestans) resistance on chromosome 5 introgressed from wild tomato solanum habrochaites. G3: GenesGenomes|Genet 3: 2131–2146

Halperin E, Stephan DA (2009) SNP imputation in association studies. Nat Biotechnol 27:349–351

Hamilton JP, Sim S-C, Stoffel K, Van Deynze A, Buell CR, Francis DM (2012) Single nucleotide polymorphism discovery in cultivated tomato via sequencing by synthesis. Plant Genome J 5:17

Han P, Lavoir A-V, Le Bot J, Amiens-Desneux E, Desneux N (2015) Nitrogen and water availability to tomato plants triggers bottom-up effects on the leafminer Tuta absoluta. Sci Rep 4:4455

Hanson PM, Yang R, Wu J, Chen J, Ledesma D, Tsou SCS, Lee T-C (2004) Variation for antioxidant activity and antioxidants in tomato. J Amer Soc Hort Sci 129:704–711

Hanssen IM, Thomma B (2010) *Pepino mosaic virus*: a successful pathogen that rapidly evolved from emerging to endemic in tomato crops. Mol Plant Pathol 11:179–189

Hanssens J, De Swaef T, Steppe K (2015) High light decreases xylem contribution to fruit growth in tomato. Plant Cell Environ 38:487–498

Harborne JB (1994) The flavonoids. Advances in research since 1986, 1st edn. Chapman Hall, London

Harvell CD, Mitchell CE, Ward JR, Altizer S, Dobson AP, Ostfeld RS, Samuel MD (2002) Climate warming and disease risks for terrestrial and marine biota. Science 296:2158–2162

Haseneyer G, Schmutzer T, Seidel M, Zhou R, Mascher M, Schön CC, Taudien S, Scholz U, Stein N, Mayer KFX, et al. (2011) From RNA-seq to large-scale genotyping—genomics resources for rye (Secale cereale L.). BMC Plant Biol 11: 131

Hayashi T, Iwata H (2010) EM algorithm for Bayesian estimation of genomic breeding values. BMC Genet 11:3

He S, Schulthess AW, Mirdita V, Zhao Y, Korzun V, Bothe R, Ebmeyer E, Reif JC, Jiang Y (2016) Genomic selection in a commercial winter wheat population. Theor Appl Genet 129:641–651

He Y (2012) Chromatin regulation of flowering. Trends Plant Sci 17:556–562
Hepler PK (2005) Calcium: a central regulator of plant growth and development. Plant Cell 17:2142–2155
Heslot N, Yang HP, Sorrells ME, Jannink JL (2012) Genomic selection in plant breeding: a comparison of models. Crop Sci 52:146–160
Hess M, Druet T, Hess A, Garrick D (2017) Fixed-length haplotypes can improve genomic prediction accuracy in an admixed dairy cattle population. Genet Sel Evol 49:54
Heuvelink E (2005) Tomatoes. CABI Publishers, Wallingford, UK
Heuvelink E, Bertin N (1994) Dry matter partitioning in a tomato crop: comparison of two simulation models. J Hort Sci 69:885–903
Hill M, Tran N (2018) MicroRNAs regulating MicroRNAs in cancer. Trends Cancer 4:465–468
Hirschi KD (2004) The calcium conundrum. Both versatile nutrient and specific signal. Plant Physiol 136:2438–2442
Ho LC (1996) The mechanism of assimilate partitioning and carbohydrate compartmentation in fruit in relation to the quality and yield of tomato. J Exp Bot 47:1239–1243
Hobson G, Grierson D (1993) Tomato. In: Biochemistry of fruit ripening. Springer, Dordrecht, pp 405–442
Hobson GE, Bedford L (1989) The composition of cherry tomatoes and its relation to consumer acceptability. J Hort Sci 64:321–329
Hospital F, Charcosset A (1997) Marker-assisted introgression of quantitative trait loci. Genetics 147:1469–1485
Hospital F, Chevalet C, Mulsant P (1992) Using markers in gene introgression breeding programs. Genetics 132:1199–1210
How Kit A, Boureau L, Stammitti-Bert L, Rolin D, Teyssier E, Gallusci P (2010) Functional analysis of SlEZ1 a tomato Enhancer of zeste (E(z)) gene demonstrates a role in flower development. Plant Mol Biol 74:201–213
Huang BE, George AW, Forrest KL, Kilian A, Hayden MJ, Morell MK, Cavanagh CR (2012) A multiparent advanced generation inter-cross population for genetic analysis in wheat. Plant Biotechnol J 10:826–839
Huang WJ, Liu HK, McCormick S, Tang WH (2014) Tomato pistil factor STIG1 promotes in vivo pollen tube growth by binding to phosphatidylinositol 3-phosphate and the extracellular domain of the pollen receptor kinase LePRK2. Plant Cell 26(6):2505–2523
Huang Z, van der Knaap E (2011) Tomato fruit weight 11.3 maps close to fasciated on the bottom of chromosome 11. Theor Appl Genet 123:465–474
Hutton SF, Scott JW, Yang WC, Sim SC, Francis DM, Jones JB (2010) Identification of QTL associated with resistance to bacterial spot race T4 in tomato. Theor Appl Genet 121:1275–1287
Ishibashi K, Masuda K, Naito S, Meshi T, Ishikawa M (2007) An inhibitor of viral RNA replication is encoded by a plant resistance gene. In: Proceedings of the national academy of sciences of the United States of America 104:13833–13838
Isidro J, Jannink J-L, Akdemir D, Poland J, Heslot N, Sorrells ME (2015) Training set optimization under population structure in genomic selection. Theor Appl Genet 128:145–158
Islam MN, Hasanuzzaman ATM, Zhang Z-F, Zhang Y, Liu T-X (2017) High level of nitrogen makes tomato plants releasing less volatiles and attracting more Bemisia tabaci (Hemiptera: Aleyrodidae). Front Plant Sci 8:466
Ito Y, Nishizawa-Yokoi A, Endo M, Mikami M, Shima Y, Nakamura N, Kotake-Nara E, Kawasaki S, Toki S (2017) Re-evaluation of the rin mutation and the role of RIN in the induction of tomato ripening. Nat Plants 3(11):866–874
Iwata H, Jannink JL (2010) Marker genotype imputation in a low-marker-density panel with a high-marker-density reference panel: accuracy evaluation in barley breeding lines. Crop Sci 50:1269–1278
Janse J, Schols M (1995) Une préférence pour un goût sucré et non farineux. Groenten Fruit 26:16–17

Jatoi SA, Fujimura T, Yamanaka S, Watanabe J, Watanabe KN, Watanabe KN (2008) Potential loss of unique genetic diversity in tomato landraces by genetic colonization of modern cultivars at a non-center of origin. Plant Breed 127:189–196

Jha UC, Bohra A, Jha R (2017) Breeding approaches and genomics technologies to increase crop yield under low-temperature stress. Plant Cell Rep 36:1–35

Jiang Y, Schmidt RH, Reif JC (2018) Haplotype-based genome-wide prediction models exploit local epistatic interactions among markers. G3: GenesGenomesGenet 8: g3.300548.2017

Jiménez-Gómez JM, Alonso-Blanco C, Borja A, Anastasio G, Angosto T, Lozano R, Martínez-Zapater JM (2007) Quantitative genetic analysis of flowering time in tomato. Genome 50:303–315

Johansson L, Haglund Å, Berglund L, Lea P, Risvik E (1999) Preference for tomatoes, affected by sensory attributes and information about growth conditions. Food Qual Prefer 10:289–298

Johnstone PR, Hartz TK, LeStrange M, Nunez JJ, Miyao EM (2005) Managing fruit soluble solids with late-season deficit irrigation in drip-irrigated processing tomato production. HortScience 40:1857–1861

Jonas E, de Koning D-J (2013) Does genomic selection have a future in plant breeding? Trends Biotechnol 31:497–504

Jones JB (1986) Survival of Xanthomonas campestris pv. vesicatoria in Florida on tomato crop residue, weeds, seeds, and volunteer tomato plants. Phytopathology 76: 430

Jones JW, Dayan E, Allen LH, Van Keulen H, Challa H (1991) A dynamic tomato growth and yield model (Tomgro). Am Soc Agri Eng 34: 663–672

Jones DA, Thomas CM, Hammondkosack KE, Balintkurti PJ, Jones JDG (1994) Isolation of the tomato cf-9 gene for resistance to Cladosporium fulvum by transposon tagging. Science 266:789–793

Kabelka E, Franchino B, Francis DM (2002) Two loci from *Lycopersicon hirsutum* LA407 confer resistance to strains of *Clavibacter michiganensis* subsp *michiganensis*. Phytopathology 92:504–510

Kamal HM, Takashina T, Egashira H, Satoh H, Imanishi S (2001) Introduction of aromatic fragrance into cultivated tomato from the "peruvianum complex". Plant Breed 120:179–181

Kang BC, Yeam I, Li HX, Perez KW, Jahn MM (2007) Ectopic expression of a recessive resistance gene generates dominant potyvirus resistance in plants. Plant Biotechnol J 5:526–536

Karimi Z, Sargolzaei M, Robinson JAB, Schenkel FS (2018) Assessing haplotype-based models for genomic evaluation in Holstein cattle. Can J Sci 1–10

Karlova R, Van Haarst JC, Maliepaard C, Van De Geest H, Bovy AG, Lammers M, Angenent GC, De Maagd RA (2013) Identification of microRNA targets in tomato fruit development using high-throughput sequencing and degradome analysis. J Exp Bot 64:1863–1878

Kawchuk LM, Hachey J, Lynch DR, Kulcsar F, Van Rooijen G, Waterer DR, Robertson A, Kokko E, Byers R, Howard RJ et al (2001) Tomato Ve disease resistance genes encode cell surface-like receptors. Proc Natl Acad Sci USA 98:6511–6515

Kazmi RH, Khan N, Willems LAJ, Van Heusden AW, Ligterink W, Hilhorst HWM (2012) Complex genetics controls natural variation among seed quality phenotypes in a recombinant inbred population of an interspecific cross between *Solanum lycopersicum* × *Solanum pimpinellifolium*. Plant Cell Environ 35:929–951

Keller M, Simm S (2018) The coupling of transcriptome and proteome adaptation during development and heat stress response of tomato pollen. BMC Genom 19:447

Kenchanmane Raju SK, Barnes AC, Schnable JC, Roston RL (2018) Low-temperature tolerance in land plants: are transcript and membrane responses conserved? Plant Sci 276:73–86

Kimbara J, Ohyama A, Chikano H, Ito H, Hosoi K, Negoro S, Miyatake K, Yamaguchi H, Nunome T, Fukuoka H et al (2018) QTL mapping of fruit nutritional and flavor components in tomato (*Solanum lycopersicum*) using genome-wide SSR markers and recombinant inbred lines (RILs) from an intra-specific cross. Euphytica 214:210

King SR, Davis AR, Zhang X, Crosby K (2010) Genetics, breeding and selection of rootstocks for Solanaceae and Cucurbitaceae. Sci Hortic (Amsterdam) 127:106–111

Kinkade MP, Foolad MR (2013) Validation and fine mapping of lyc12.1, a QTL for increased tomato fruit lycopene content. Theor Appl Genet 126:2163–2175
Kissoudis C, Chowdhury R, van Heusden S, van de Wiel C, Finkers R, Visser RGF, Bai Y, van der Linden G (2015) Combined biotic and abiotic stress resistance in tomato. Euphytica 202:317–332
Klay I, Gouia S, Liu M, Mila I, Khoudi H, Bernadac A et al (2018) Ethylene Response Factors (ERF) are differentially regulated by different abiotic stress types in tomato plants. Plant Sci 274:137–145
Klee HJ (2010) Improving the flavor of fresh fruits: genomics, biochemistry, and biotechnology. New Phytol 187:44–56
Klee HJ (2013) Purple tomatoes: Longer lasting, less disease, and better for you. Curr Biol 23:R520–R521
Klee HJ, Tieman DM (2013) Genetic challenges of flavor improvement in tomato. Trends Genet 29:257–262
Klee HJ, Tieman DM (2018) The genetics of fruit flavour preferences. Nat Rev Genet 19:347–356
Klein RJ, Zeiss C, Chew EY, Tsai J-Y, Sackler RS, Haynes C, Henning AK, Paul SanGiovanni J, Mane SM, Mayne ST et al (2005) Complement factor H polymorphism in age-related macular degeneration. Science 308:385–389
Kooke R, Kruijer W, Bours R, Becker F, Kuhn A, van de Geest H, Buntjer J, Doeswijk T, Guerra J, Bouwmeester H et al (2016) Genome-wide association mapping and genomic prediction elucidate the genetic architecture of morphological traits in arabidopsis. Plant Physiol 170:2187–2203
Korte A, Vilhjálmsson BJ, Segura V, Platt A, Long Q, Nordborg M (2012) A mixed-model approach for genome-wide association studies of correlated traits in structured populations. Nat Genet 44:1066–1071
Kover PX, Valdar W, Trakalo J, Scarcelli N, Ehrenreich IM, Purugganan MD, Durrant C, Mott R (2009) A multiparent advanced generation inter-cross to fine-map quantitative traits in *Arabidopsis thaliana*. PLoS Genet 5:e1000551
Kramer M, Sanders R, Bolkan H, Waters C, Sheeny RE, Hiatt WR (1992) Postharvest evaluation of transgenic tomatoes with reduced levels of polygalacturonase: processing, firmness and disease resistance. Postharv Biol Technol 1(3):241–255
Kramer MG, Redenbaugh K (1994) Commercialization of a tomato with an antisense polygalacturonase gene: the FLAVR SAVR? tomato story. Euphytica 79:293–297
Krieger U, Lippman ZB, Zamir D (2010) The flowering gene SINGLE FLOWER TRUSS drives heterosis for yield in tomato. Nat Genet 42:459–463
Kromdijk J, Bertin N, Heuvelink E, Molenaar J, de Visser PHB, Marcelis LFM, Struik PC (2013) Crop management impacts the efficiency of QTL detection and use—case study of fruit load x QTL interactions. J Exp Bot. https://doi.org/10.1093/jxb/ert365
Kropff MJ, Haverkort AJ, Aggarwal PK, Kooman PL (1995) Using systems approaches to design and evaluate ideotypes for specific environments. In: Bouma J, Bouman BAM, Luyten JC, Zandstra HG (eds) Eco-regional approaches for sustainable land use and food production. Kluwer Academic Publ, Dordrecht, Netherlands, pp 417–435
Kumar M, Ashok I, Chandrawat S (2016) Gene pyramiding: an overview. Intl J Curr Res Biosci Plant Biol. https://doi.org/10.20546/ijcrbp.2016.307.004
Kusmec A, Srinivasan S, Nettleton D, Schnable PS (2017) Distinct genetic architectures for phenotype means and plasticities in Zea mays. Nat Plants 3:715–723
Kyriacou MC, Rouphael Y, Colla G, Zrenner R, Schwarz D (2017) Vegetable grafting: the implications of a growing agronomic imperative for vegetable fruit quality and nutritive value. Front Plant Sci 8:741
Labate JA, Grandillo S, Fulton T, Muños S, Caicedo AL, Peralta I, Ji Y, Chetelat RT, Scott JW, Gonzalo MJ et al (2007) Tomato. In: Kole C (ed) Genome mapping and molecular breeding in plants, vol 5. Vegetables. Springer, Berlin, pp 1–125
Lanfermeijer FC, Warmink J, Hille J (2005) The products of the broken *Tm-2* and the durable *Tm-2(2)* resistance genes from tomato differ in four amino acids. J Exp Bot 56:2925–2933

Lang Z, Wang Y, Tang K, Tang D, Datsenka T, Cheng J, Zhang Y, Handa AK, Zhu JK (2017) Critical roles of DNA demethylation in the activation of ripening-induced genes and inhibition of ripening-repressed genes in tomato fruit. Proc Natl Acad Sci USA 114(22):E4511–E4519

Lapidot M, Karniel U, Gelbart D, Fogel D, Evenor D, Kutsher Y, Makhbash Z, Nahon S, Shlomo H, Chen L, Reuveni M, Levin I (2015) A novel route controlling begomovirus resistance by the messenger RNA surveillance factor *Pelota*. PLoS Genetics 11

Larbat R, Olsen KM, Slimestad R, Løvdal T, Bénard C, Verheul M, Bourgaud F, Robin C, Lillo C (2012) Influence of repeated short-term nitrogen limitations on leaf phenolics metabolism in tomato. Phytochemistry 77:119–128

Laterrot H (1996) Twenty-one near isogenic lines in Moneymaker type with different genes for disease resistances. Rep Tomato Genet Coop 46:34

Laterrot H (2000) Disease resistance in tomato: practical situation. Acta Physiol Plant 22:328–331

Laterrot H, Moretti A (1989) Linkage between *Pto* and susceptibility to fenthion. Tomato Genet Coop Rep 39:21–22

Le Nguyen K, Grondin A, Courtois B, Gantet P (2018) Next-generation sequencing accelerates crop gene discovery. Trends Plant Sci 24:263–274

Le LQ, Lorenz Y, Scheurer S, Fötisch K, Enrique E, Bartra J, Biemelt S, Vieths S, Sonnewald U (2006) Design of tomato fruits with reduced allergenicity by dsRNAi-mediated inhibition of ns-LTP (Lyc e 3) expression. Plant Biotechnol J 4(2):231–242

Lecompte F, Abro MA, Nicot PC (2010) Contrasted responses of Botrytis cinerea isolates developing on tomato plants grown under different nitrogen nutrition regimes. Plant Pathol 59:891–899

Lecompte F, Nicot PC, Ripoll J, Abro MA, Raimbault AK, Lopez-Lauri F, Bertin N (2017) Reduced susceptibility of tomato stem to the necrotrophic fungus *Botrytis cinerea* is associated with a specific adjustment of fructose content in the host sugar pool. Ann Bot 119:931–943

Lecomte L, Saliba-Colombani V, Gautier A, Gomez-Jimenez MC, Duffé P, Buret M, Causse M (2004a) Fine mapping of QTLs for the fruit architecture and composition in fresh market tomato, on the distal region of the long arm of chromosome 2. Mol Breed 13:1–14

Lecomte L, Duffé P, Buret M, Servin B, Hospital F, Causse M (2004b) Marker-assisted introgression of 5 QTLs controlling fruit quality traits into three tomato lines revealed interactions between QTLs and genetic backgrounds. Theor Appl Genet 109:658–668

Lee DR (1990) A unidirectional water flux model of fruit growth. Can J Bot 68:1286–1290

Lee JM, Oh CS, Yeam I (2015) Molecular markers for selecting diverse disease resistances in tomato breeding programs. Plant Breed Biotechnol 3:308–322

Lee JT, Prasad V, Yang PT, Wu JF, David Ho TH, Charng YY, Chan MT (2003) Expression of Arabidopsis CBF1 regulated by an ABA/stress inducible promoter in transgenic tomato confers stress tolerance without affecting yield. Plant Cell Environ 26(7):1181–1190

Lee SY, Luna-Guzman I, Chang S, Barrett DM, Guinard JX (1999) Relating descriptive analysis and instrumental texture data of processed diced tomatoes. Food Qual Pref 10:447–455

Lefebvre V, Boissot N, Gallois J-L (2018) Host plant resistance to pests and pathogens, the genetic leverage in integrated pest and disease management. In: Gullino ML, Albajes R, Nicot P, van Lenteren JC (eds) Pest and disease management in greenhouse crops. Developments in Plant Pathology. Springer International Publishing, Cham

Length F (2011) Genetic diversity in 14 tomato (*Lycopersicon esculentum* Mill.) varieties in Nigerian markets by RAPD-PCR technique. Afr J Biotechnol 10(11):4961–4967

Letort V, Mahe P, Cournede PH, De Reffye P, Courtois B (2008) Quantitative genetics and functional-structural plant growth models: Simulation of quantitative trait loci detection for model parameters and application to potential yield optimization. Ann Bot-London 101:1243–1254

Levin I, Gilboa N, Yeselson E, Shen S, Schaffer AA (2000) Fgr, a major locus that modulates the fructose to glucose ratio in mature tomato fruits. Theor Appl Genet 100:256–262

Li YM, Gabelman WH (1990) Inheritance of calcium use efficiency in tomatoes grown under low-calcium stress. J Am Soc Hortic Sci 115:835–838

Li J, Liu L, Bai Y, Zhang P, Finkers R, Du Y et al (2011) Seedling salt tolerance in tomato. Euphytica 178:403–414

Li T, Yang X, Yu Y, Si X, Zhai X, Zhang H, Dong W, Gao C, Xu C (2018) Domestication of wild tomato is accelerated by genome editing. Nat Biotechnol 36:1160–1163
Lin KH, Yeh WL, Chen HM, Lo HF (2010) Quantitative trait loci influencing fruit-related characteristics of tomato grown in high-temperature conditions. Euphytica 174:119–135
Lin T, Zhu G, Zhang J, Xu X, Yu Q, Zheng Z, Zhang Z, Lun Y, Li S, Wang X et al (2014) Genomic analyses provide insights into the history of tomato breeding. Nat Genet 46:1220–1226
Lindemose S, O'Shea C, Jensen M, Skriver K, Lindemose S, O'Shea C et al (2013) Structure, function and networks of transcription factors involved in abiotic stress responses. Int J Mol Sci 14:5842–5878
Lippman ZB, Zamir D (2007) Heterosis: revisiting the magic. Trends Genet 23:60–66
Liu Z, Alseekh S, Brotman Y, Zheng Y, Fei Z, Tieman DM, Giovannoni JJ, Fernie AR, Klee HJ (2016b) Identification of a Solanum pennellii chromosome 4 fruit flavor and nutritional quality-associated metabolite QTL. Front Plant Sci 7:1–15
Liu H, Genard M, Guichard S, Bertin N (2007) Model-assisted analysis of tomato fruit growth in relation to carbon and water fluxes. J Exp Bot 58:3567–3580
Liu J, Van Eck J, Cong B, Tanksley SD (2002) A new class of regulatory genes underlying the cause of pear-shaped tomato fruit. Proc Natl Acad Sci USA 99:13302–13306
Liu HJ, Yan J (2019) Crop genome-wide association study: a harvest of biological relevance. Plant J 97:8–18
Liu H, Yu C, Li H, Ouyang B, Wang T, Zhang J et al (2015) Overexpression of SHDHN, a dehydrin gene from Solanum habrochaites enhances tolerance to multiple abiotic stresses in tomato. Plant Sci 231:198–211
Liu M, Yu H, Zhao G et al (2017) Profiling of drought-responsive microRNA and mRNA in tomato using high-throughput sequencing. BMC Genomics 18:481
Liu Y, Zhou T, Ge H, Pang W, Gao L, Ren L et al (2016a) SSR mapping of QTLs conferring cold tolerance in an interspecific cross of tomato. Intl J Genom 2016:1–6
Lobit P, Génard M, Soing P, Habib R (2006) Modelling malic acid accumulation in fruits: relationships with organic acids, potassium, and temperature. J Exp Bot 57:1471–1483
Lobit P, Génard M, Wu BH, Soing P, Habib R (2003) Modelling citrate metabolism in fruits: responses to growth and temperature. J Exp Bot 54:2489–2501
Lü P, Yu S, Zhu N, Chen Y-R, Zhou B, Pan Y, Tzeng D, Fabi JP, Argyris J, Garcia-Mas J et al. (2018) Genome encode analyses reveal the basis of convergent evolution of fleshy fruit ripening. Nat Plants 1
Luo J (2015) Metabolite-based genome-wide association studies in plants. Curr Opin Plant Biol 24:31–38
Maayan Y, Pandaranayaka EPJ, Srivastava DA, Lapidot M, Levin I, Dombrovsky A, Harel A (2018) Using genomic analysis to identify tomato *Tm-2* resistance-breaking mutations and their underlying evolutionary path in a new and emerging tobamovirus. Arch Virol 163:1863–1875
Mackay IJ, Bansept-Basler P, Barber T, Bentley AR, Cockram J, Gosman N, Greenland AJ, Horsnell R, Howells R, O'Sullivan DM et al (2014) An eight-parent multiparent advanced generation inter-cross population for winter-sown wheat: creation, properties, and validation. G3: GenesGenomGenet 4: 1603–1610
Madhavi DL, Salunkhe DK (1998) Handbook of vegetable science and technology. In: Salunkhe DK, Kadam SS (eds) Production, composition, storage, and processing, New York, USA. https://doi.org/10.1201/9781482269871
Malundo TMM, Shewfelt RL, Scott JW (1995) Flavor quality of fresh tomato (*Lycopersicon esculentum* Mill.) as affected by sugar and acid levels. Postharv BiolTechnol 6:103–110
Manavella PA, Hagmann J, Ott F, Laubinger S, Franz M, Macek B, Weigel D (2012) Fast-forward genetics identifies plant CPL phosphatases as regulators of miRNA processing factor HYL1. Cell 151:859–870
Mangin B, Rincent R, Rabier CE, Moreau L, Goudemand-Dugue E (2019) Training set optimization of genomic prediction by means of EthAcc. PLoS ONE 14:e0205629

Mangin B, Thoquet P, Olivier J, Grimsley NH (1999) Temporal and multiple quantitative trait loci analyses of resistance to bacterial wilt in tomato permit the resolution of linked loci. Genetics 151:1165–1172

Manning K, Tör M, Poole M, Hong Y, Thompson AJ, King GJ, Giovannoni JJ, Seymour GB (2006) A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. Nat Genet 38:948–952

Mao L, Begum D, Chuang H, Budiman MA, Szymkowiak EJ, Irish EE, Wing RA (2000) JOINTLESS is a MADS-box gene controlling tomato flower abscissionzone development. Nature 406:910–913

Marchini J, Howie B (2010) Genotype imputation for genome-wide association studies. Nat Rev Genet 11:499–511

Marques de Carvalho L, Benda ND, Vaughan MM, Cabrera AR, Hung K, Cox T, Abdo Z, Allen LH, Teal PE (2015) *Mi-1*-mediated nematode resistance in tomatoes is broken by short-term heat stress but recovers over time. J Nematol 47:133–140

Marschner H (1983) General introduction to the mineral nutrition of plants. In: Lauchli A, Bieleski R (eds) Inorganic plant nutrition. Springer, Berlin, pp 5–60

Martin GB, Brommonschenkel SH, Chunwongse J, Frary A, Ganal MW, Spivey R, Wu T, Earle ED, Tanksley SD, Sipvey R et al (1993) Map-based cloning of a protein kinase gene conferring disease resistance in tomato. Science (80-) 262:1432–1436

Martin GB, Frary A, Wu T, Brommonschenkel S, Chunwongse J, Earle ED, Tanksley SD (1994) A member of the tomato *Pto* gene family confers sensitivity to fenthion resulting in rapid cell death. Plant Cell 6:1543–1552

Martre P, Bertin N, Salon C, Génard M (2011) Modelling the size and composition of fruit, grain and seed by process-based simulation models. New Phytolt Tansley Review 191:601–618

Martre P, Quilot-Turion B, Luquet D, Ould-Sidi M-M, Chenu K, Debaeke P (2015) Model-assisted phenotyping and ideotype design. In: Sadras V, Calderini D (eds) Crop physiology: applications for genetic improvement and agronomy. Academic Press, London, pp 349–373

Mazzucato A, Cellini F, Bouzayen M, Zouine M, Mila I, Minoia S, Petrozza A, Picarella ME, Ruiu F, Carriero F (2015) A TILLING allele of the tomato Aux/IAA9 gene offers new insights into fruit set mechanisms and perspectives for breeding seedless tomatoes. Mol Breed 35:22

Mazzucato A, Papa R, Bitocchi E, Mosconi P, Nanni L, Negri V, Picarella ME, Siligato F, Soressi GP, Tiranti B et al (2008) Genetic diversity, structure and marker-trait associations in a collection of Italian tomato (*Solanum lycopersicum* L.) landraces. Theor Appl Genet 116:657–669

Mboup M, Fischer I, Lainer H, Stephan W (2012) Trans-species polymorphism and Allele-Specific expression in the CBF gene family of wild tomatoes. Mol Biol Evol 29:3641–3652

McCormick S, Niedermeyer J, Fry J, Barnason A, Horsch R, Fraley R (1986) Leaf disc transformation of cultivated tomato (*L. esculentum*) using *Agrobacterium tumefaciens*. Plant Cell Rep 5(2):81–84

McCouch SR, Wright MH, Tung C-W, Maron LG, McNally KL, Fitzgerald M, Singh N, DeClerck G, Agosto-Perez F, Korniliev P et al (2016) Open access resources for genome-wide association mapping in rice. Nat Commun 7:10532

McGlasson WB, Last JH, Shaw KJ, Meldrum SK (1987) Influence of the non-ripening mutant rin and nor on the aroma of tomato fruits. HortScience 22:632–634

Meena YK, Khurana DS, Singh K (2018) Towards enhanced low temperature stress tolerance in tomato : an approach. J Environ Biol. https://doi.org/10.22438/jeb/39/4/MRN-590

Megraw M, Baev V, Rusinov V, Jensen ST, Kalantidis K, Hatzigeorgiou AG (2006) MicroRNA promoter element discovery in Arabidopsis. RNA 12:1612–1619

Menda N, Semel Y, Peled D, Eshed Y, Zamir D (2004) *Insilico* screening of a saturated mutation library of tomato. Plant J 38:861–872

Menda N, Strickler SR, Edwards JD, Bombarely A, Dunham DM, Martin GB, Mejia L, Hutton SF, Havey MJ, Maxwell DP et al (2014) Analysis of wild-species introgressions in tomato inbreds uncovers ancestral origins. BMC Plant Biol 14:287

Mendell JT, Olson EN (2012) MicroRNAs in stress signaling and human disease. Cell 148:1172–1187
Meng C, Yang D, Ma X, Zhao W, Liang X, Ma N, Meng Q (2016) Suppression of tomato SlNAC1 transcription factor delays fruit ripening. J Plant Physiol 193:88–96
Meng FJ, Xu XY, Huang FL, Li JF (2010) Analysis of genetic diversity in cultivated and wild tomato varieties in Chinese market by RAPD and SSR. Agri Sci China 9:1430–1437
Messeguer R, Ganal M, de Vicente MC, Young ND, Bolkan H, Tanksley SD (1991) High resolution RFLP map around the root knot nematode resistance gene (*Mi*) in tomato. Theor Appl Genet 82:529–536
Meuwissen THE, Hayes BJ, Goddard ME (2001) Prediction of total genetic value using genome-wide dense marker maps. Genetics 157:1819–1829
Migault V, Pallas B, Costes E (2017) Combining genome-wide information with a functional structural plant model to simulate 1-year-old apple tree architecture. Front Plant Sci. https://doi.org/10.3389/fpls.2016.02065
Miller JC, Tanksley SD (1990) RFLP analysis of phylogenetic relationships and genetic variation in the genus Lycopersicon. Theor Appl Genet 80:437–448
Milligan SB, Bodeau J, Yaghoobi J, Kaloshian I, Zabel P, Williamson VM (1998) The root knot nematode resistance gene *Mi* from tomato is a member of the leucine zipper, nucleotide binding, leucine-rich repeat family of plant genes. Plant Cell 10:1307–1319
Milner S et al (2011) Bioactivities of glycoalkaloids and their aglycones from Solanum species. J Agri Food Chem 59:3454–3484
Minamikawa MF, Nonaka K, Kaminuma E, Kajiya-Kanegae H, Onogi A, Goto S, Yoshioka T, Imai A, Hamada H, Hayashi T et al (2017) Genome-wide association study and genomic prediction in citrus: Potential of genomics-assisted breeding for fruit quality traits. Sci Rep 7:4721
Minoïa S, Bendahmane A, Piron F, Salgues A, Moretti A, Caranta C, Piednoir E, Nicolaï M, Zamir D (2010) An induced mutation in tomato eIF4E leads to immunity to two potyviruses. PLoS ONE 5:e11313
Minoia S, Cellini F, Bendahmane A, D'Onofrio O, Petrozza A, Carriero F, Piron F, Mosca G, Sozio G (2010) A new mutant genetic resource for tomato crop improvement by TILLING technology. BMC Res Notes. https://doi.org/10.1186/1756-0500-3-69
Mirnezhad M, Romero-Gonzalez RR, Leiss KA, Choi YH, Verpoorte R, Klinkhamer PG (2010) Metabolomic analysis of host plant resistance to thrips in wild and cultivated tomatoes. Phytochem Analys 21(1):110–117
Mirouze M, Paszkowski J (2011) Epigenetic contribution to stress adaptation in plants. Curr Opin Plant Biol 14:267–274
Mitchell J, Shennan C, Grattan S (1991) Developmental-changes in tomato fruit composition in response to water deficit and salinity. Physiol Plant 83:177–185
Mohorianu I, Schwach F, Jing R, Lopez-Gomollon S, Moxon S, Szittya G, Sorefan K, Moulton V, Dalmay T (2011) Profiling of short RNAs during fleshy fruit development reveals stage-specific sRNAome expression patterns. Plant J 67:232–246
Molgaard P, Ravn H (1988) Evolutionary aspects of caffeoyl ester distribution in dicotyledons. Phytochemistry 27:2411–2421
Monforte AJ, Asíns MJ, Carbonell EA (1996) Salt tolerance in Lycopersicon species. IV. Efficiency of marker-assisted selection for salt tolerance improvement. Theor Appl Genet 93–93:765–772
Monforte AJ, Asíns MJ, Carbonell EA (1997a) Salt tolerance in Lycopersicon species VI. Genotype-by-salinity interaction in quantitative trait loci detection: constitutive and response QTLs. Theor Appl Genet 95:706–713
Monforte AJ, Asíns MJ, Carbonell EA (1997b) Salt tolerance in Lycopersicon species. V. Does genetic variability at quantitative trait loci affect their analysis? Theor Appl Genet 95:284–293
Monforte AJ, Tanksley SD (2000) Fine mapping of a quantitative trait locus (QTL) from *Lycopersicon hirsutum* chromosome 1 affecting fruit characteristics and agronomic traits: breaking linkage among QTLs affecting different traits and dissection of heterosis for yield. Theor Appl Genet 100:471–479

Montesinos-López OA, Montesinos-López A, Crossa J, Toledo FH, Pérez-Hernández O, Eskridge KM, Rutkoski J (2016) A genomic Bayesian MULTI-TRAIT AND MULTI-ENVIRONMENT MODEL. G3: GenesGenomGenet 6: 2725–2744

Moxon S, Jing R, Szittya G, Schwach F, Rusholme Pilcher RL, Moulton V, Dalmay T (2008) Deep sequencing of tomato short RNAs identifies microRNAs targeting genes involved in fruit ripening. Genome Res 18:1602–1609

Mu Q, Huang Z, Chakrabarti M, Illa-Berenguer E, Liu X, Wang Y, Ramos A, van der Knaap E (2017) Fruit weight is controlled by cell size regulator encoding a novel protein that is expressed in maturing tomato fruits. PLoS Genet 13:e1006930

Muir SR, Collins GJ, Robinson S, Hughes S, Bovy A, Ric De Vos CH, van Tunen AJ, Verhoeyen ME (2001) Overexpression of petunia chalcone isomerase in tomato results in fruit containing increased levels of flavonols. Nat Biotechnol 19:470–474

Mueller LA, Tanksley SD, Giovannoni JJ, van Eck J, Stack S, Choi D, Kim BD, Chen M, Cheng Z, Li C, Ling H, Xue Y, Seymour G, Bishop G, Bryan G, Sharma R, Khurana J, Tyagi A, Chattopadhyay D, Singh NK, Stiekema W, Lindhout P, Jesse T, Lankhorst RK, Bouzayen M, Shibata D, Tabata S, Granell A, Botella MA, Giuliano G, Frusciante L, Causse M, Zamir D (2005) The tomato sequencing project, the first cornerstone of the International Solanaceae Project (SOL). Comp Funct Genomics 6(3):153–158

Müller BSF, Neves LG, de Almeida Filho JE, Resende MFR, Muñoz PR, dos Santos PET, Filho EP, Kirst M, Grattapaglia D (2017) Genomic prediction in contrast to a genome-wide association study in explaining heritable variation of complex growth traits in breeding populations of Eucalyptus. BMC Genom 18:524

Munns R, Gilliham M (2015) Salinity tolerance of crops - what is the cost? New Phytol 208:668–673

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681

Muños S, Ranc N, Botton E, Bérard A, Rolland S, Duffé P, Carretero Y, Le Paslier MC, Delalande C, Bouzayen M, Brunel D, Causse M (2011) Increase in tomato locule number is controlled by two single-nucleotide polymorphisms located near WUSCHEL. Plant Physiol 156(4):2244–2254

Mutshinda CM, Sillanpää MJ (2010) Extended Bayesian LASSO for multiple quantitative trait loci mapping and unobserved phenotype prediction. Genetics 186:1067–1075

Nadeem M, Li J, Wang M, Shah L, Lu S, Wang X, Ma C, Nadeem M, Li J, Wang M et al (2018) Unraveling field crops sensitivity to heat stress: mechanisms, approaches, and future prospects. Agronomy 8:128

Nakazato T, Warren DL, Moyle LC (2010) Ecological and geographic modes of species divergence in wild tomatoes. Amer J Bot 97:680–693

Navarro JM, Flores P, Carvajal M, Martinez V (2005) Changes in quality and yield of tomato fruit with ammonium, bicarbonate and calcium fertilisation under saline conditions. J Hort Sci Biotechnol 80:351–357

Naves ER, de Ávila Silva L, Sulpice R, Araújo WL, Nunes-Nesi A, Peres LE, Zsögön A (2019) Capsaicinoids: pungency beyond capsicum. Trends Plant Sci 24:109–120

Nawaz MA, Imtiaz M, Kong Q, Cheng F, Ahmed W, Huang Y et al (2016) Grafting: a technique to modify ion accumulation in horticultural crops. Front Plant Sci 7:1457

Nekrasov V, Wang C, Win J, Lanz C, Weigel D, Kamoun S (2017) Rapid generation of a transgene-free powdery mildew resistant tomato by genome deletion. Sci Rep 7:482

Nesbitt TC, Tanksley SD (2002) Comparative sequencing in the genus Lycopersicon: implications for the evolution of fruit size in the domestication of cultivated tomatoes. Genetics 162:365–379

Nombela G, Williamson VM, Muniz M (2003) The root-knot nematode resistance gene *Mi-1.2* of tomato is responsible for resistance against the whitefly *Bemisia tabaci*. Mol Plant-Microbe Interact 16:645–649

Nuruddin MM, Madramootoo CA, Dodds GT (2003) Effects of water stress at different growth stages on greenhouse tomato yield and quality. HortScience 38:1389–1393

Ofner I, Lashbrooke J, Pleban T, Aharoni A, Zamir D (2016) Solanum pennellii backcross inbred lines (BILs) link small genomic bins with tomato traits. Plant J 87:151–160

Ohlson EW, Ashrafi H, Foolad MR (2018) Identification and mapping of late blight resistance quantitative trait loci in tomato accession PI 163245. Plant Genome 11
Ohlson EW, Foolad MR (2016) Genetic analysis of resistance to tomato late blight in Solanum pimpinellifolium accession PI 163245. Plant Breed 135:391–398
Okabe Y, Asamizu E, Saito T, Matsukura C, Ariizumi T, Brès C, Rothan C, Mizoguchi T, Ezura H (2011) Tomato TILLING technology: development of a reverse genetics tool for the efficient isolation of mutants from Micro-Tom mutant libraries. Plant Cell Physiol 52:1994–2005
Okello RCO, Heuvelink E, de Visser PHB, Struik PC, Marcelis LFM (2015) What drives fruit growth? Funct Plant Biol 42:817–827
Oliver JE, Whitfield AE (2016) The genus tospovirus: emerging bunyaviruses that threaten food security. In: Enquist LW (ed) Annu Rev Virol 3:101–124
Ongom PO, Ejeta G (2017) Mating design and genetic structure of a multi-parent advanced generation intercross (MAGIC) population of Sorghum (*Sorghum bicolor* (L.) Moench). G3 Genes|Genomes|Genetics 8:331–341
Ori N, Eshed Y, Paran I, Presting G, Aviv D, Tanksley S, Zamir D, Fluhr R (1997) The I2C family from the wilt disease resistance locus I2 belongs to the nucleotide binding, leucine-rich repeat superfamily of plant resistance genes. Plant Cell (The) 9:521–532
Osorio S, Ruan Y-L, Fernie AR (2014) An update on source-to-sink carbon partitioning in tomato. Front Plant Sci 5:516
Ould-Sidi M-M, Lescourret F (2011) Model-based design of innovative cropping systems: state of the art and new prospects. AgronSustain Dev 31(3):571–588
Overy SA, Walker HJ, Malone S, Howard TP, Baxter CJ, Sweetlove LJ, Hill SA, Quick WP (2004) Application of metabolite profiling to the identification of traits in a population of tomato introgression lines. J Exp Bot 56:287–296
Pailles Y, Ho S, Pires IS, Tester M, Negrão S, Schmöckel SM (2017) Genetic diversity and population structure of two tomato species from the Galapagos Islands. Front Plant Sci 8:138
Panthee DR, Piotrowski A, Ibrahem R (2017) Mapping Quantitative Trait Loci (QTL) for resistance to late blight in tomato. Int J Mol Sci 18 (7). pii: E1589. https://doi.org/10.3390/ijms18071589
Papadopoulos I, Rendig VV (1983) Interactive effects of salinity and nitrogen on growth and yield of tomato plants. Plant Soil 73:47–57
Paran I, Goldman I, Tanksley SD, Zamir D (1995) Recombinant inbred lines for genetic mapping in tomato. Theor Appl Genet 90:542–548
Park T, Casella G (2008) The Bayesian lasso. J Amer Stat Assoc 103:681–686
Park YH, West MA, St. Clair DA (2004) Evaluation of AFLPs for germplasm fingerprinting and assessment of genetic diversity in cultivars of tomato (*Lycopersicon esculentum* L.). Genome 47:510–518
Pasaniuc B, Rohland N, McLaren PJ, Garimella K, Zaitlen N, Li H, Gupta N, Neale BM, Daly MJ, Sklar P et al (2012) Extremely low-coverage sequencing and imputation increases power for genome-wide association studies. Nat Genet 44:631–635
De Pascale S, Maggio A, Fogliano V, Ambrosino P, Ritieni A (2001) Irrigation with saline water improves carotenoids content and antioxidant activity of tomato. J Hort Sci Biotechnol 76:447–453
Pascual L, Desplat N, Huang BE, Desgroux A, Bruguier L, Bouchet JP, Le QH, Chauchard B, Verschave P, Causse M (2015) Potential of a tomato MAGIC population to decipher the genetic control of quantitative traits and detect causal variants in the resequencing era. Plant Biotechnol J 13:565–577
Patanè C, Cosentino SL (2010) Effects of soil water deficit on yield and quality of processing tomato under a mediterranean climate. Agri Water Manag 97:131–138
Paterson AH, Damon S, Hewitt JD, Zamir D, Rabinowitch HD, Loncoln SE, Lander ES, Tanksley SD (1991) Mendelian factors underlying quantitative traits in tomato: comparison across species, generations, and environments. Genetics 127:181–197

Paterson AH, DeVerna JW, Lanini B, Tanksley SD (1990) Fine mapping of quantitative trait loci using selected overlapping recombinant chromosomes, in an interspecies cross of tomato. Genetics 124:735–742

Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335:721–726

Pattison RJ, Csukasi F, Zheng Y, Fei Z, van der Knaap E, Catalá C (2015) Comprehensive tissue-specific transcriptome analysis reveals distinct regulatory programs during early tomato fruit development. Plant Physiol 168(4):1684–1701

Peralta IE, Knapp S, Spooner DM (2005) New species of wild tomatoes (Solanum Section Lycopersicon: Solanaceae) from Northern Peru. Syst Bot 30:424–434

Pertuzé RA, Ji Y, Chetelat RT (2003) Comparative linkage map of the *Solanum lycopersicoides* and *S. sitiens* genomes and their differentiation from tomato. Genome 45:1003–1012

Petró-Turza M (1986) Flavor of tomato and tomato products. Food Rev Intl 2:309–351

Pettigrew WT (2008) Potassium influences on yield and quality production for maize, wheat, soybean and cotton. Physiol Plant 133:670–681

Philouze J (1991) Description of isogenic lines, except for one, or two, monogenically controlled morphological traits in tomato, *Lycopersicon esculentum* Mill. Euphytica 56:121–131

Pillen K, Ganal MW, Tanksley SD (1996) Construction of a high-resolution genetic map and YAC-contigs in the tomato *Tm-2a* region. Theor Appl Genet 93:228–233

Piron F, Nicolai M, Minoia S, Piednoir E, Moretti A, Salgues A, Zamir D, Caranta C, Bendahmane A (2010) An induced mutation in tomato *eIF4E* leads to immunity to two potyviruses. PloS One 5

Pnueli L, Carmel-Goren L, Hareven D, Gutfinger T, Alvarez J, Ganal M, Zamir D, Lifschitz E (1998) The SELF-PRUNING gene of tomato regulates vegetative to reproductive switching of sympodial meristems and is the ortholog of CEN and TFL1. Development 125(11):1979–1989

Poiroux-Gonord F, Bidel LPR, Fanciullino A-L, Gautier H, Lauri-Lopez F, Urban L (2010) Health benefits of vitamins and secondary metabolites of fruits and vegetables and prospects to increase their concentrations by agronomic approaches. J Agri Food Chem 58:12065–12082

Poland JA, Balint-Kurti PJ, Wisser RJ, Pratt RC, Nelson RJ (2009) Shades of gray: the world of quantitative disease resistance. Trends Plant Sci 14:21–29

Prudent M, Lecomte A, Bouchet JP, Bertin N, Causse M, Génard M (2011) Combining eco-physiological modelling and quantitative trait loci analysis to identify key elementary processes underlying tomato fruit sugar concentration. J Exp Bot 62:907–919

Qi LS, Larson MH, Gilbert LA, Doudna JA, Weissman JS, Arkin AP, Lim WA (2013) Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. Cell 152(5):1173–1183

Quadrana L, Almeida J, Asís R, Duffy T, Dominguez PG, Bermúdez L, Conti G, Corrêa da Silva JV, Peralta IE, Colot V et al (2014) Natural occurring epialleles determine vitamin E accumulation in tomato fruits. Nat Commun 5:4027

Quilot B, Kervella J, Genard M, Lescourret F (2005) Analysing the genetic control of peach fruit quality through an ecophysiological model combined with a QTL approach. J Exp Bot 56:3083–3092

Quilot-Turion B, Génard M, Valsesia P, Memmah M-M (2016) Optimization of allelic combinations controlling parameters of a Peach quality model. Front Plant Sci 7:1873

Quilot-Turion B, Ould-Sidi M-M, Kadrani A, Hilgert N, Génard M, Lescourret F (2012) Optimization of parameters of the 'Virtual Fruit' model to design peach genotype for sustainable production systems. Eur J Agron 42:34–48

Quinet M, Kinet J-M, Lutts S (2011) Flowering response of the uniflora: blind: self-pruning and jointless: uniflora: self-pruning tomato (*Solanum lycopersicum*) triple mutants. Physiol Plant 141:166–176

Rached M, Pierre B, Yves G, Matsukura C, Ariizumi T, Ezura H et al (2018) Differences in blossom-end rot resistance in tomato cultivars is associated with total ascorbate rather than calcium concentration in the distal end part of fruits *per se* Hortic J 87:372–381

Rajasekaran LR, Aspinall D, Paleg LG (2000) Physiological mechanism of tolerance of *Lycopersicon spp.* exposed to salt stress. Can J Plant Sci 80:151–159

Rajewsky N (2006) microRNA target predictions in animals. Nat Genet 38:S8–S13

Rambla JL, Tikunov YM, Monforte AJ, Bovy AG, Granell A (2014) The expanded tomato fruit volatile landscape. J Exp Bot 65:4613–4623

Ramstein GP, Jensen SE, Buckler ES (2018) Breaking the curse of dimensionality to identify causal variants in Breeding 4. Theor Appl Genet 132:559–567

Ranc N, Muños S, Xu J, Le Paslier M-C, Chauveau A, Bounon R, Rolland S, Bouchet J-P, Brunel D, Causse M (2012) Genome-wide association mapping in tomato (*Solanum lycopersicum*) is possible using genome admixture of *Solanum lycopersicum* var. *cerasiforme*. G3: GenesGenomesGenet 2: 853–864

Ranjan A, Budke JM, Rowland SD, Chitwood DH, Kumar R, Carriedo L et al (2016) eQTL regulating transcript levels associated with diverse biological processes in tomato. Plant Physiol 172(1):328–340

Rao ES, Kadirvel P, Symonds RC, Ebert AW (2013) Relationship between survival and yield related traits in Solanum pimpinellifolium under salt stress. Euphytica 190:215–228

Rasmussen S, Barah P, Suarez-Rodriguez MC, Bressendorff S, Friis P, Costantino P, Bones AM, Nielsen HB, Mundy J (2013) Transcriptome responses to combinations of stresses in arabidopsis. Plant Physiol 161:1783–1794

Rengel Z (1992) The role of calcium in salt toxicity. Plant Cell Environ 15:625–663

Reymond M, Muller B, Leonardi A, Charcosset A, Tardieu F (2003) Combining quantitative trait loci analysis and an ecophysiological model to analyze the genetic variability of the responses of maize leaf growth to temperature and water deficit. Plant Physiol 131:664–675

Rick CM, Chetelat RT (1995) Utilization of related wild species for tomato improvement. In: FernandezMunoz R, Cuartero J, GomezGuillamon ML (eds) First international symposium on solanaceae for fresh market. Acta Hort 412:21–38

Ripoll J, Urban L, Brunel B, Bertin N (2016) Water deficit effects on tomato quality depend on fruit developmental stage and genotype. J Plant Physiol 190:26–35

Ripoll J, Urban L, Staudt M, Lopez-Lauri F, Bidel LPR, Bertin N (2014) Water shortage and quality of fleshy fruits—making the most of the unavoidable. J Exp Bot 65:4097–4117

Rivero RM, Mestre TC, Mittler R, Rubio F, Garcia-Sanchez F, Martinez V (2014) The combined effect of salinity and heat reveals a specific physiological, biochemical and molecular response in tomato plants. Plant Cell Environ 37:1059–1073

Robbins MD, Masud MAT, Panthee DR, Gardner RG, Francis DM, Stevens MR (2010) Marker-assisted selection for coupling phase resistance to *Tomato spotted wilt virus* and *Phytophthora infestans* (Late Blight) in tomato. HortScience 45:1424–1428

Robert VJM, West MAL, Inai S, Caines A, Arntzen L, Smith JK, St Clair DA (2001) Marker-assisted introgression of blackmold resistance QTL alleles from wild L*ycopersicon cheesmanii* to cultivated tomato (*L. esculentum*) and evaluation of QTL phenotypic effects. Mol Breed 8:217–233

Rodríguez GR, Muños S, Anderson C, Sim S-C, Michel A, Causse M, Gardener BBM, Francis D, van der Knaap E (2011) Distribution of SUN, OVATE, LC, and FAS in the tomato germplasm and the relationship to fruit shape diversity. Plant Physiol 156:275–285

Rodríguez-Leal D, Lemmon ZH, Man J, Bartlett ME, Lippman ZB (2017) Engineering quantitative trait variation for crop improvement by genome editing. Cell 171:470–480

Rogers K, Chen X (2013) Biogenesis, turnover, and mode of action of plant MicroRNAs. Plant Cell 25:2383–2399

Ronen G, Cohen M, Zamir D, Hirschberg J (1999) Regulation of carotenoid biosynthesis during tomato fruit development: expression of the gene for lycopene epsilon-cyclase is down-regulated during ripening and is elevated in the mutant Delta. Plant J17:341–351

Rosales MA, Rubio-Wilhelmi MM, Castellano R, Castilla N, Ruiz JM, Romero L (2007) Sucrolytic activities in cherry tomato fruits in relation to temperature and solar radiation. Sci Hort (Amsterdam) 113:244–249

Rosental L, Perelman A, Nevo N, Toubiana D, Samani T, Batushansky A, Sikron N, Saranga Y, Fait A (2016) Environmental and genetic effects on tomato seed metabolic balance and its association with germination vigor. BMC Genom 17:1047

Rossi M, Goggin FL, Milligan SB, Kaloshian I, Ullman DE, Williamson VM (1998) The nematode resistance gene *Mi* of tomato confers resistance against the potato aphid. Proc Natl Acad Sci USA 95:9750–9754

Rothan C, Diouf I, Causse M (2019) Trait discovery and editing in tomato. Plant J 97:73–90

Rousseaux MC, Jones CM, Adams D, Chetelat R, Bennett A, Powell A (2005) QTL analysis of fruit antioxidants in tomato using *Lycopersicon pennellii* introgression lines. Theor Appl Genet 111:1396–1408

Ruan Y-L, Patrick JW, Bouzayen M, Osorio S, Fernie AR (2012) Molecular regulation of seed and fruit set. Trends Plant Sci 17:656–665

Ruffel S, Gallois JL, Lesage ML, Caranta C (2005) The recessive potyvirus resistance gene *pot-1* is the tomato orthologue of the pepper *pvr2-eIF4E* gene. Mol Genet Genom 274:346–353

Ruggieri V, Francese G, Sacco A, Alessandro AD, Rigano MM, Parisi M, Milone M, Cardi T, Mennella G, Barone A (2014) An association mapping approach to identify favourable alleles for tomato fruit quality breeding. BMC Plant Biol 14:1–15

Sacco A, Di Matteo A, Lombardi N, Trotta N, Punzo B, Mari A, Barone A (2013) Quantitative trait loci pyramiding for fruit quality traits in tomato. Mol Breed 31(1):217–222

Sahu KK, Chattopadhyay D (2017) Genome-wide sequence variations between wild and cultivated tomato species revisited by whole genome sequence mapping. BMC Genom 18:430

Sainju UM, Dris R, Singh B (2003) Mineral nutrition of tomato. Food Agri Environ 1:176–183

Saliba-Colombani V, Causse M, Langlois D, Philouze J, Buret M (2001) Genetic analysis of organoleptic quality in fresh market tomato: 1. Mapping QTLs for physical and chemical traits. Theor Appl Genet 102:259–272

Sallam A, Martsch R (2015) Association mapping for frost tolerance using multi-parent advanced generation inter-cross (MAGIC) population in faba bean (*Vicia faba* L.). Genetica 143:501–514

Salmeron JM, Oldroyd GE, Rommens CM, Scofield SR, Kim H-S, Lavelle DT, Dahlbeck D, Staskawicz BJ (1996) Tomato Prf is a member of the leucine-rich repeat class of plant disease resistance genes and lies embedded within the Pto Kinase gene cluster. Cell 86:123–133

Sanei M, Chen X (2015) Mechanisms of microRNA turnover. Curr Opin Plant Biol 27:199–206

Sarlikioti V, de Visser PHB, Buck-Sorlin GH, Marcelis LFM (2011) How plant architecture affects light absorption and photosynthesis in tomato: towards an ideotype for plant architecture using a functional–structural plant model. Ann Bot 108(6):1065–1073

Sato S, Tabata S, Hirakawa H et al (2012) The tomato genome sequence provides insights into fleshy fruit evolution. Nature 485:635–641

Sauvage C, Rau A, Aichholz C, Chadoeuf J, Sarah G, Ruiz M, Santoni S, Causse M, David J, Glémin S (2017) Domestication rewired gene expression and nucleotide diversity patterns in tomato. Plant J 91:631–645

Sauvage C, Segura V, Bauchet G, Stevens R, Do PT, Nikoloski Z, Fernie AR, Causse M (2014) Genome-wide association in tomato reveals 44 candidate loci for fruit metabolic traits. Plant Physiol 165:1120–1132

Schachtman DP, Shin R (2007) Nutrient sensing and signaling: NPKS. Annu Rev Plant Biol 58:47–69

Schaffer AA, Levin I, Oguz I, Petreikov M, Cincarevsky F, Yeselson Y, Shen S, Gilboa N, Bar M (2000) ADPglucose pyrophosphorylase activity and starch accumulation in immature tomato fruit: the effect of a *Lycopersicon hirsutum*-derived introgression encoding for the large subunit. Plant Sci 152:135–144

Schauer N, Semel Y, Roessner U, Gur A, Balbo I, Carrari F, Pleban T, Perez-Melis A, Bruedigam C, Kopka J et al (2006) Comprehensive metabolic profiling and phenotyping of interspecific introgression lines for tomato improvement. Nat Biotechnol 24:447–454

Schauer N, Zamir D, Fernie AR (2005) Metabolic profiling of leaves and fruit of wild species tomato: a survey of the *Solanum lycopersicum* complex. J Exp Bot 56:297–307

Scheben A, Batley J, Edwards D (2017) Genotyping-by-sequencing approaches to characterize crop genomes: choosing the right tool for the right application. Plant Biotechnol J 15:149–161

Schijlen EG, de Vos CR, Martens S, Jonker HH, Rosin FM, Molthoff JW, Tikunov YM, Angenent GC, van Tunen AJ, Bovy AG (2007) RNA interference silencing of chalcone synthase, the first step in the flavonoid biosynthesis pathway, leads to parthenocarpic tomato fruits. Plant Physiol 144(3):1520–1530

Scholberg JMS, Locascio SJ (1999) Growth response of snap bean and tomato as affected by salinity and irrigation method. HortScience 34:259–264

Segura V, Vilhjálmsson BJ, Platt A, Korte A, Seren Ü, Long Q, Nordborg M (2012) An efficient multi-locus mixed-model approach for genome-wide association studies in structured populations. Nat Genet 44:825–830

Semel Y, Nissenbaum J, Menda N, Zinder M, Krieger U, Issman N, Pleban T, Lippman Z, Gur A, Zamir D (2006) Overdominant quantitative trait loci for yield and fitness in tomato. Proc Natl Acad Sci USA 103:12981–12986

Semel Y, Schauer N, Roessner U, Zamir D, Fernie AR (2007) Metabolite analysis for the comparison of irrigated and non-irrigated field grown tomato of varying genotype. Metabolomics 3:289–295

Shahlaei A, Torabi S, Khosroshahli M (2014) Efficiacy of SCoT and ISSR marekers in assesment of tomato (*Lycopersicum esculentum* Mill.) genetic diversity. Intl J Biosci 5:14–22

Shalit A, Rozman A, Goldshmidt A, Alvarez JP, Bowman JL, Eshed Y, Lifschitz E (2009) The flowering hormone florigen functions as a general systemic regulator of growth and termination. Proc Natl Acad Sci USA 106:8392–8397

Shammai A, Petreikov M, Yeselson Y, Faigenboim A, Moy-Komemi M, Cohen S, Cohen D, Besaulov E, Efrati A, Houminer N et al (2018) Natural genetic variation for expression of a SWEET transporter among wild species of *Solanum lycopersicum* (tomato) determines the hexose composition of ripening tomato fruit. Plant J 96:343–357

Sharada MS, Kumari A, Pandey AK, Sharma S, Sharma P, Sreelakshmi Y, Sharma R (2017) Generation of genetically stable transformants by *Agrobacterium* using tomato floral buds. Plant Cell Tiss Org Cult 129(2):299–312

Shimatani Z, Kashojiya S, Takayama M, Terada R, Arazoe T, Ishii H, Teramura H, Yamamoto T, Komatsu H, Miura K, Ezura H (2017) Targeted base editing in rice and tomato using a CRISPR-Cas9 cytidine deaminase fusion. Nature Biotechnol 35(5):441–443

Shinozaki Y, Nicolas P, Fernandez-Pozo N, Ma Q, Evanich DJ, Shi Y, Xu Y, Zheng Y, Snyder SI, Martin LBB et al (2018) High-resolution spatiotemporal transcriptome mapping of tomato fruit development and ripening. Nat Commun 9:364

Sim S-C, Van Deynze A, Stoffel K, Douches DS, Zarka D, Ganal MW, Chetelat RT, Hutton SF, Scott JW, Gardner RG, et al. (2012a) High-density SNP genotyping of tomato (*Solanum lycopersicum* L.) reveals patterns of genetic variation due to breeding. PLoS One 7:e45520

Sim S-C, Durstewitz G, Plieske J, Wieseke R, Ganal MW, van Deynze A, Hamilton JP, Buell CR, Causse M, Wijeratne S et al (2012b) Development of a large snp genotyping array and generation of high-density genetic maps in tomato. PLoS One. https://doi.org/10.1371/journal.pone.0040563

Sim S-C, Robbins MD, Van Deynze A, Agee M, Francis DM (2010) Population structure and genetic differentiation associated with breeding history and selection in tomato (*Solanum lycopersicum* L.). Heredity (Edinb) 106:927–935

Sim SC, Robbins MD, Wijeratne S, Wang H, Yang WC, Francis DM (2015) Association analysis for bacterial spot resistance in a directionally selected complex breeding population of tomato. Phytopathology 105:1437–1445

Simons G, Groenendijk J, Wijbrandi J, Reijans M, Groenen J, Diergaarde P, Van der Lee T, Bleeker M, Onstenk J, de Both M, Haring M, Mes J, Cornelissen B, Zabeau M, Vos P (1998) Dissection

of the Fusarium I2 gene cluster in tomato reveals six homologs and one active gene copy. Plant Cell (The) 10:1055–1068
Smart CD, Tanksley SD, Mayton H, Fry WE (2007) Resistance to *Phytophthora infestans* in *Lycopersicon pennellii*. Plant Dis 91:1045–1049
Smirnoff N, Wheeler GL (2000) Ascorbic acid in plants: biosynthesis and function. Crit Rev Biochem Mol Biol 35:291–314
Smith DL, Abbott JA, Gross KC (2002) Down-regulation of tomato β-galactosidase 4 results in decreased fruit softening. Plant Physiol 129(4):1755–1762
Soyk S, Lemmon ZH, Oved M et al (2017a) Bypassing negative epistasis on yield in tomato imposed by a domestication gene. Cell 169:1142–1155
Soyk S, Müller NA, Park SJ, Schmalenbach I, Jiang K, Hayama R, Zhang L, Van Eck J, Jiménez-Gómez JM, Lippman ZB (2017b) Variation in the flowering gene SELF PRUNING 5G promotes day-neutrality and early yield in tomato. Nat Genet 49:162–168
Spano R, Mascia T, Kormelink R, Gallitelli D (2015) Grafting on a non-transgenic tolerant tomato variety confers resistance to the infection of a *Sw5*-breaking strain of *tomato spotted wilt virus* via RNA silencing. PloS One 10
Spindel J, Begum H, Akdemir D, Virk P, Collard B, Redoña E, Atlin G, Jannink JL, McCouch SR (2015) Genomic selection and association mapping in rice (Oryza sativa): effect of trait genetic architecture, training population composition, marker number and statistical model on accuracy of rice genomic selection in elite, tropical rice breeding lines. PLoS Genet 11:1–25
Stamova BS, Chetelat RT (2000) Inheritance and genetic mapping of *cucumber mosaic virus* resistance introgressed from *Lycopersicon chilense* into tomato. TheorAppl Genet 101:527–537
Stevens MA (1986) Inheritance of tomato fruit quality components. Plant Breed Rev 4:273–311
Stevens MA, Kader AA, Albright M (1979) Potential for increasing tomato flavor via increased sugar and acid content. J Amer Soc Hort Sci 104:40–42
Stevens MA, Kader AA, Albright-Holton M (1977) Intercultivar variation in composition of locular and pericarp portions of fresh market tomatoes. J Amer Soc Hort Sci 102:689–692
Stevens MR, Lamb EM, Rhoads DD (1995) Mapping the *Sw-5* locus for *tomato spotted wilt virus*-resistance in tomatoes using RAPD and RFLP analyses. Theor Appl Genet 90:451–456
Stikic R, Popovic S, Srdic M, Savic D, Jovanovic Z, Zdravkovic J (2003) Partial root drying (PRD): a new technique for growing plants that saves water and improves the quality of fruit. Bulg J Plant Physiol 164–171
Stricker SH, Köferle A, Beck S (2017) From profiles to function in epigenomics. Nat Rev Genet 18:51–66
Struik PC, Yin XY, de Visser P (2005) Complex quality traits: now time to model. Trends Plant Sci 10:513–516
Suliman-Pollatschek S, Kashkush K, Shats H, Hillel J, Lavi U (2002) Generation and mapping of AFLP, SSRs and SNPs in *Lycopersicon esculentum*. Cell Mol Biol Lett 7:583–597
Sun J, Poland JA, Mondal S, Crossa J, Juliana P, Singh RP, Rutkoski JE, Jannink J-L, Crespo-Herrera L, Velu G et al (2019) High-throughput phenotyping platforms enhance genomic selection for wheat grain yield across populations and cycles in early stage. Theor Appl Genet 1–16
Sun X, Gao Y, Li H, Yang S, Liu Y (2015) Over-expression of SlWRKY39 leads to enhanced resistance to multiple stress factors in tomato. J Plant Biol 58:52–60
Suzuki N, Rivero RM, Shulaev V, Blumwald E, Mittler R (2014) Abiotic and biotic stress combinations. New Phytol 203:32–43
Tadmor Y, Fridman E, Gur A, Larkov O, Lastochkin E, Ravid U, Zamir D, Lewinsohn E (2002) Identification of malodorous, a wild species allele affecting tomato aroma that was selected against during domestication. J Agri Food Chem 50:2005–2009
Takken FLW, Thomas CM, Joosten M, Golstein C, Westerink N, Hille J, Nijkamp HJJ, De Wit P, Jones JDG (1999) A second gene at the tomato *Cf-4* locus confers resistance to *Cladosporium fulvum* through recognition of a novel avirulence determinant. Plant J 20:279–288

Takken FLW, Schipper D, Nijkamp HJJ, Hille J (1998) Identification and Ds-tagged isolation of a new gene at the Cf-4 locus of tomato involved in disease resistance to Cladosporium fulvum race 5. Plant J 14:401–411

Tam SM, Mhiri C, Vogelaar A, Kerkveld M, Pearce SR, Grandbastien MA (2005) Comparative analyses of genetic diversities within tomato and pepper collections detected by retrotransposon-based SSAP, AFLP and SSR. Theor Appl Genet 110:819–831

Tanksley SD (2004) The genetic, developmental, and molecular bases of fruit size in tomato and shape variation. Plant Cell 16:181–190

Tanksley SD, Ganal MW, Prince JP, De Vicente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB et al (1992) High density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141–1160

Tanksley SD, Grandillo S, Fulton TM, Zamir D, Eshed Y, Petiard V, Lopez J, Beck-Bunn T (1996) Advanced backcross QTL analysis in a cross between an elite processing line of tomato and its wild relative *L. pimpinellifolium*. Theor Appl Genet 92:213–224

Tanksley SD, Nelson JC (1996) Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theor Appl Genet 92:191–203

Tardieu F (2003) Virtual plants: modelling as a tool for the genomics of tolerance to water deficit. Trends Plant Sci 8:9–14

Tashkandi M, Ali Z, Aljedaani F, Shami A, Mahfouz MM (2018) Engineering resistance against *Tomato yellow leaf curl virus* via the CRISPR/Cas9 system in tomato. Plant Signal Behav 13

Taudt A, Colomé-Tatché M, Johannes F (2016) Genetic sources of population epigenomic variation. Nat Rev Genet 17:319–332

The 100 Tomato Genome Sequencing Consortium (2014) Exploring genetic variation in the tomato (*Solanum* section *Lycopersicon*) clade by whole-genome sequencing. Plant J 80:136–148

The 100 Tomato Genome Sequencing Consortium (2014) Exploring genetic variation in the tomato (*Solanum* section *Lycopersicon*) clade by whole-genome sequencing. Plant J 80:136–148

The 1000 Genomes Project Consortium (2010) A map of human genome variation from population-scale sequencing. Nature 467:1061–1073

The 1001 Genomes Consortium (2016) 1,135 genomes reveal the global pattern of polymorphism in *Arabidopsis thaliana*. Cell 166: 481–491

The 1001 Genomes Consortium (2016) 1,135 genomes reveal the global pattern of polymorphism in *Arabidopsis thaliana*. Cell 166: 481–491

The 3000 rice genomes project (2014) The 3,000 rice genomes project. Gigascience 3: 7

The UK10K Consortium (2015) The UK10K project identifies rare variants in health and disease. Nature 526:82–89

Thoen MPM, Davila Olivas NH, Kloth KJ, Coolen S, Huang PP, Aarts MGM, Bac-Molenaar JA, Bakker J, Bouwmeester HJ, Broekgaarden C et al (2017) Genetic architecture of plant stress resistance: multi-trait genome-wide association mapping. New Phytol 213:1346–1362

Tieman D, Bliss P, McIntyre LMM, Blandon-Ubeda A, Bies D, Odabasi AZZ, Rodríguez GRR, Van Der Knaap E, Taylor MGG, Goulet C et al (2012) The chemical interactions underlying tomato flavor preferences. Curr Biol 22:1035–1039

Tieman D, Taylor M, Schauer N, Fernie AR, Hanson AD, Klee HJ (2006) Tomato aromatic amino acid decarboxylases participate in synthesis of the flavor volatiles 2-phenylethanol and 2-phenylacetaldehyde. Proc Natl Acad Sci USA 103:8287–8292

Tieman D, Zhu G, Resende MFR, Lin T, Nguyen C, Bies D, Rambla JL, Beltran KSO, Taylor M, Zhang B et al (2017) A chemical genetic roadmap to improved tomato flavor. Science (80-) 355:391–394

Tieman DM, Handa AK (1994) Reduction in pectin methylesterase activity modifies tissue integrity and cation levels in ripening tomato (*Lycopersicon esculentum* Mill.) fruits. Plant Physiol 106(2):429–36

Tikunov Y, Lommen A, Vos CHR, de Verhoeven HA, Bino RJ, Hall RD, Bovy AG (2005) A novel approach for nontargeted data analysis for metabolomics. Large-scale profiling of tomato fruit volatiles. Plant Physiol 139:1125–1137

Tikunov YM, Molthoff J, de Vos RCH, Beekwilder J, van Houwelingen A et al (2013) Non-smoky glycosyltransferase1 prevents the release of smoky aroma from tomato fruit. Plant Cell 25(8):3067–3078

Tranchida-Lombardo V, Aiese Cigliano R, Anzar I, Landi S, Palombieri S, Colantuono C, Bostan H, Termolino P, Aversano R, Batelli G et al (2018) Whole-genome re-sequencing of two Italian tomato landraces reveals sequence variations in genes associated with stress tolerance, fruit quality and long shelf-life traits. DNA Res 25:149–160

Tuna AL, Kaya C, Ashraf M, Altunlu H, Yokas I, Yagmur B (2007) The effects of calcium sulphate on growth, membrane stability and nutrient uptake of tomato plants grown under salt stress. Environ Exp Bot 59:173–178

Turina M, Kormelink R, Resende RO (2016) Resistance to tospoviruses in vegetable crops: epidemiological and molecular aspects. In: Leach JE, Lindow S (eds) Annu Rev Phytopathol 54:347–371

Uluisik S, Chapman NH, Smith R, Poole M, Adams G, Gillis RB, Besong TM, Sheldon J, Stiegelmeyer S, Perez L, Samsulrizal N (2016) Genetic improvement of tomato by targeted control of fruit softening. Nature Biotechnol 34(9):950

Usadel B, Chetelat R, Koren S, Maumus F, Fernie AR, Aury J-M, Maß J, Schmidt MH-W, Denton AK, Wormit A et al (2017) De novo assembly of a new Solanum pennellii accession using nanopore sequencing. Plant Cell 29:2336–2348

Vakalounakis DJ, Laterrot H, Moretti A, Ligoxigakis EK, Smardas K (1997) Linkage between *Frl* (*Fusarium oxysporum* f sp *radicis-lycopersici* resistance) and *Tm-2* (*tobacco mosaic virus resistance-2*) loci in tomato (*Lycopersicon esculentum*). Ann Appl Biol 130:319–323

van Berloo R, Stam P (1998) Marker-assisted selection in autogamous RIL populations: a simulation study. Theor Appl Genet 96:147–154

van Berloo R, Stam P (1999) Comparison between marker-assisted selection and phenotypical selection in a set of *Arabidopsis thaliana* recombinant inbred lines. Theor Appl Genet 98:113–118

Van Berloo R, Zhu A, Ursem R, Verbakel H, Gort G, van Eeuwijk FA (2008) Diversity and linkage disequilibrium analysis within a selected set of cultivated tomatoes. Theor Appl Genet 117:89–101

van der Knaap E, Tanksley SD (2003) The making of a bell pepper-shaped tomato fruit: identification of loci controlling fruit morphology in Yellow Stuffer tomato. Theor Appl Genet 107:139–147

van Eeuwijk Fred A, Bustos-Korts D, Millet EJ, Boer MP, Kruijer W, Thompson A et al (2019) Modelling strategies for assessing and increasing the effectiveness of new phenotyping techniques in plant breeding. Plant Sci 282:23–39

Vargas-Ponce O, Pérez-Álvarez LF, Zamora-Tavares P, Rodríguez A (2011) Assessing genetic diversity in mexican husk tomato species. Plant Mol Biol Rep 29:733–738

Veillet F, Perrot L, Chauvin L, Kermarrec M-P, Guyon-Debast A, Chauvin J-E, Nogué F, Mazier M (2019) Transgene-free genome editing in tomato and potato plants using *Agrobacterium*-mediated delivery of a CRISPR/Cas9 cytidine base editor. Intl J Mol Sci 20(2):402

Venter F (1977) Solar radiation and vitamin C content of tomato fruits. Acta Hortic 58:121–127

Verkerke W, Janse J, Kersten M (1998) Instrumental measurement and modelling of tomato fruit taste. Acta Hort 199–206

Verlaan MG, Hutton SF, Ibrahem RM, Kormelink R, Visser RGF, Scott JW, Edwards JD, Bai YL (2013) The *Tomato Yellow Leaf Curl Virus* Resistance Genes *Ty-1* and *Ty-3* Are Allelic and Code for DFDGD-Class RNA-Dependent RNA Polymerases. PLoS Genetics 9

Villalta I, Bernet GP, Carbonell EA, Asins MJ (2007) Comparative QTL analysis of salinity tolerance in terms of fruit yield using two solanum populations of F7 lines. Theor Appl Genet 114:1001–1017

Víquez-Zamora M, Vosman B, van de Geest H, Bovy A, Visser RGF, Finkers R, van Heusden AW (2013) Tomato breeding in the genomics era: insights from a SNP array. BMC Genom 14:354

Vos P, Simons G, Jesse T, Wijbrandi J, Heinen L, Hogers R, Frijters A, Groenendijk J, Diergaarde P, Reijans M, Fierens-Onstenk J, de Both M, Peleman J, Liharska T, Hontelez J, Zabeau M (1998) The tomato *Mi-1* gene confers resistance to both root-knot nematodes and potato aphids. Nat Biotechnol 16:1365–1369

Vrebalov J, Ruezinsky D, Padmanabhan V, White R, Medrano D, Drake R, Schuch W, Giovannoni J (2002) A MADS-box gene necessary for fruit ripening at the tomato ripening-inhibitor (rin) locus. Science (80-) 296:343–346

Wahid A, Gelani S, Ashraf M, Foolad MR (2007) Heat tolerance in plants: an overview. Environ Exp Bot 61:199–223

Wang D, Salah El-Basyoni I, Stephen Baenziger P, Crossa J, Eskridge KM, Dweikat I (2012) Prediction of genetic values of quantitative traits with epistatic effects in plant breeding populations. Heredity (Edinb) 109:313–319

Wang DR, Agosto-Pérez FJ, Chebotarov D, Shi Y, Marchini J, Fitzgerald M, McNally KL, Alexandrov N, McCouch SR (2018) An imputation platform to enhance integration of rice genetic resources. Nat Commun 9:3519

Wang JF, Ho FI, Truong HTH, Huang SM, Balatero CH, Dittapongpitch V, Hidayati N (2013a) Identification of major QTLs associated with stable resistance of tomato cultivar 'Hawaii 7996' to *Ralstonia solanacearum*. Euphytica 190:241–252

Wang K, Li M, Hakonarson H (2010) ANNOVAR: Functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 38:e164

Wang L, Song X, Gu L, Li X, Cao S, Chu C, Cui X, Chen X, Cao X (2013b) NOT2 proteins promote polymerase II-dependent transcription and interact with multiple MicroRNA biogenesis factors in Arabidopsis. Plant Cell 25:715–727

Wang R, Tavano ECDR, Lammers M, Martinelli AP, Angenent GC, de Maagd RA (2019) Re-evaluation of transcription factor function in tomato fruit development and ripening with CRISPR/Cas9-mutagenesis. Sci Rep 8:1696

Wang Y, Wu W-H (2015) Genetic approaches for improvement of the crop potassium acquisition and utilization efficiency. Curr Opin Plant Biol 25:46–52

Wang Z, Gerstein M, Snyder M (2009) RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet 10:57–63

Waters AJ, Makarevitch I, Noshay J, Burghardt LT, Hirsch CN, Hirsch CD, Springer NM (2017) Natural variation for gene expression responses to abiotic stress in maize. Plant J 89:706–717

Wells T, Ward JL, Corol DI, Baker JM, Gerrish C, Michael H, Seymour GB, Fraser PD and Bramley PM (2013) Metabolite profiling of introgression lines of Solanum habrochaites using targeted and non-targeted approaches reveals novel quantitative trait loci. PhD Max Planck Institute Postdam, Germany, 149 p

Wilkins KA, Matthus E, Swarbreck SM, Davies JM (2016) Calcium-mediated abiotic stress signaling in roots. Front Plant Sci 7:1296

Willits MG, Kramer CM, Prata RT, De Luca V, Potter BG, Steffens JC, Graser G (2005) Utilization of the genetic resources of wild species to create a nontransgenic high flavonoid tomato. J Agri Food Chem 53:1231–1236

Won SY, Yumul RE, Chen X (2014) Small RNAs in plants. Molecular biology. Springer, New York, pp 95–127

Xiao H, Jiang N, Schaffner E, Stockinger EJ, van der Knaap E (2008) A retrotransposon-mediated gene duplication underlies morphological variation of tomato fruit. Science 319:1527–1530

Xie Z, Allen E, Fahlgren N, Calamar A, Givan SA, Carrington JC (2005) Expression of Arabidopsis MIRNA genes. Plant Physiol 138:2145–2154

Xu J, Driedonks N, Rutten MJM, Vriezen WH, de Boer GJ, Rieu I (2017a) Mapping quantitative trait loci for heat tolerance of reproductive traits in tomato (*Solanum lycopersicum*). Mol Breed 37:58

Xu J, Ranc N, Muños S, Rolland S, Bouchet J-PP, Desplat N, Le Paslier M-CC, Liang Y, Brunel D, Causse M (2013) Phenotypic diversity and association mapping for fruit quality traits in cultivated tomato and related species. Theor Appl Genet 126:567–581

Xu J, Wolters-Arts M, Mariani C, Huber H, Rieu I (2017b) Heat stress affects vegetative and reproductive performance and trait correlations in tomato (*Solanum lycopersicum*). Euphytica 213:156

Xu WF, Shi WM, Yan F (2012) Temporal and tissue-specific expression of tomato 14-3-3 gene family in response to phosphorus deficiency. Pedosphere 22:735–745

Yamaguchi H, Ohnishi J, Saito A, Ohyama A, Nunome T, Miyatake K, Fukuoka H (2018) An NB-LRR gene, TYNBS1, is responsible for resistance mediated by the Ty-2 Begomovirus resistance locus of tomato. Theoret Appl Genet 131:1345–1362

Yamamoto E, Matsunaga H, Onogi A, Kajiya-Kanegae H, Minamikawa M, Suzuki A, Shirasawa K, Hirakawa H, Nunome T, Yamaguchi H et al (2016) A simulation-based breeding design that uses whole-genome prediction in tomato. Sci Rep 6:19454

Yamamoto E, Matsunaga H, Onogi A, Ohyama A, Miyatake K, Yamaguchi H, Nunome T, Iwata H, Fukuoka H (2017) Efficiency of genomic selection for breeding population design and phenotype prediction in tomato. Heredity (Edinb) 118:202–209

Yang D-Y, Li M, Ma N-N, Yang X-H, Meng Q-W (2017) Tomato SlGGP-LIKE gene participates in plant responses to chilling stress and pathogenic infection. Plant Physiol Biochem 112:218–226

Yang X, Caro M, Hutton SF, Scott JW, Guo Y, Wang X, Rashid MH, Szinay D, de Jong H, Visser RGF et al (2014) Fine mapping of the tomato yellow leaf curl virus resistance gene Ty-2 on chromosome 11 of tomato. Mol Breed 34:749–760

Yasmeen A, Mirza B, Inayatullah S, Safdar N, Jamil M, Ali S, Choudhry MF (2009) In planta transformation of tomato. Plant Mol Biol Rep 27(1):20–28

Ye J, Wang X, Hu T, Zhang F, Wang B, Li C, Yang T, Li H, Lu Y, Giovannoni JJ et al (2017) An InDel in the promoter of Al-ACTIVATED MALATE TRANSPORTER9 selected during tomato domestication determines fruit malate contents and aluminum tolerance. Plant Cell 29:2249–2268

Yin X, Kropff MJ, Stam P (1999) The role of ecophysiological models in QTL analysis: the example of specific leaf area in barley. Heredity 82:415–421

You C, Cui J, Wang H, Qi X, Kuo L-Y, Ma H, Gao L, Mo B, Chen X (2017) Conservation and divergence of small RNA pathways and microRNAs in land plants. Genome Biol 18:158

Young ND, Tanksley SD (1989) RFLP analysis of the size of chromosomal segments retained around the Tm-2 locus of tomato during backcross breeding. Theor Appl Genet 77:353–359

Yu B, Bi L, Zheng B, Ji L, Chevalier D, Agarwal M, Ramachandran V, Li W, Lagrange T, Walker JC et al (2008) The FHA domain proteins DAWDLE in Arabidopsis and SNIP1 in humans act in small RNA biogenesis. Proc Natl Acad Sci USA105: 10073–10078

Yu Y, Jia T, Chen X (2017) The 'how' and 'where' of plant microRNAs. New Phytol 216:1002–1017

Zamir D (2001) Improving plant breeding with exotic genetic libraries. Nat Rev Genet 2:3–9

Zanor MI, Rambla JL, Chaïb J, Steppa A, Medina A, Granell A, Fernie AR, Causse M (2009) Metabolic characterization of loci affecting sensory attributes in tomato allows an assessment of the influence of the levels of primary metabolites and volatile organic contents. J Exp Bot 60:2139–2154

Zegbe-Domínguez J, Behboudian M, Lang A, Clothier B (2003) Deficit irrigation and partial root-zone drying maintain fruit dry mass and enhance fruit quality in 'Petopride' processing tomato (*Lycopersicon esculentum*, Mill.). Sci Hort (Amsterdam) 98:505–510

Zhang B, Tieman DM, Chen J, Xu Y, Chen K, Fei Z, Giovannoni J, Klee HJ (2016) Loss of tomato flavor quality during chilling is associated with reduced expression of volatile biosynthetic genes and a transient alteration in DNA methylation. Proc Natl Acad Sci USA113:12580–12584

Zhang C, Liu L, Wang X, Vossen J, Li G, Li T, Zheng Z, Gao J, Guo Y, Visser RGF et al (2014) The Ph-3 gene from Solanum pimpinellifolium encodes CC-NBS-LRR protein conferring resistance to *Phytophthora infestans*. Theor Appl Genet 127:1353–1364

Zhang J, Zhao J, Liang Y, Zou Z (2016b) Genome-wide association-mapping for fruit quality traits in tomato. Euphytica 207:439–451

Zhang J, Zhao J, Xu Y, Liang J, Chang P, Yan F, Li M, Liang Y, Zou Z (2015a) Genome-wide association mapping for tomato volatiles positively contributing to tomato flavor. Front Plant Sci 6:1042

Zhang S, Xie M, Ren G, Yu B (2013) CDC5, a DNA binding protein, positively regulates posttranscriptional processing and/or transcription of primary microRNA transcripts. Proc Natl Acad Sci USA 110:17588–17593

Zhang Y, Butelli E, Alseekh S, Tohge T, Rallapalli G, Luo J, Kawar PG, Hill L, Santino A, Fernie AR, Martin C (2015b) Multi-level engineering facilitates the production of phenylpropanoid compounds in tomato. Nat Commun 6:8635

Zhang Z, Guo X, Ge C, Ma Z, Jiang M, Li T, Koiwa H, Yang SW, Zhang X (2017) KETCH1 imports HYL1 to nucleus for miRNA biogenesis in Arabidopsis. Proc Natl Acad Sci USA 114:4011–4016

Zhao C, Liu B, Piao S, Wang X, Lobell DB, Huang Y, Huang M, Yao Y, Bassu S, Ciais P et al (2017) Temperature increase reduces global yields of major crops in four independent estimates. Proc Natl Acad Sci USA114: 9326–9331

Zhao J, Sauvage C, Zhao J, Bitton F, Bauchet G, Liu D, Huang S, Tieman DM, Klee HJ, Causse M (2019) Meta-analysis of genome-wide association studies provides insights into genetic control of tomato flavor. Nat Commun 10:1534

Zhao X, Liu Y, Liu X, Jiang J (2018) Comparative transcriptome profiling of two tomato genotypes in response to potassium-deficiency stress. Int J Mol Sci 19:2402

Zhong S, Fei Z, Chen Y, Zheng Y, Huang M, Vrebalov J, McQuinn R, Gapper N, Liu B, Xiang J et al (2013) Single-base resolution methylomes of tomato fruit development reveal epigenome modifications associated with ripening. Nat Biotechnol 31:154–159

Zhou R, Wu Z, Cao X, Jiang F (2015) Genetic diversity of cultivated and wild tomatoes revealed by morphological traits and SSR markers. Genet Mol Res 14:13868–13879

Zhou R, Yu X, Ottosen C-O, Rosenqvist E, Zhao L, Wang Y, Yu W, Zhao T, Wu Z (2017) Drought stress had a predominant effect over heat stress on three tomato cultivars subjected to combined stress. BMC Plant Biol 17:24

Zhu G, Gou J, Klee H, Huang S (2019) Next-gen approaches to flavor-related metabolism. Ann Rev Plant Biol 70:187–212

Zhu G, Wang S, Huang Z, Zhang S, Liao Q et al (2018) Rewiring of the fruit metabolome in tomato breeding. Cell 172:249–261

Zhuang K, Kong F, Zhang S, Meng C, Yang M, Liu Z, Wang Y, Ma N, Meng Q (2019) Whirly1 enhances tolerance to chilling stress in tomato via protection of photosystem II and regulation of starch degradation. New Phytol 221:1998–2012

Zsögön A, Čermák T, Naves ER, Notini MM, Edel KH, Weinl S, Freschi L, Voytas DF, Kudla J, Peres LE (2018) De novo domestication of wild tomato using genome editing. Nat Biotechnol 36:1211–1216

Zsögön A, Cermak T, Voytas D, Pereira Peres LE (2017) Genome editing as a tool to achieve the crop ideotype and de novo domestication of wild relatives: case study in tomato. Plant Sci 256:120–130

Zuo J, Fu D, Zhu Y, Qu G, Tian H, Zhai B, Ju Z, Gao C, Wang Y, Luo Y et al (2013) SRNAome parsing yields insights into tomato fruit ripening control. Physiol Plant 149:540–553

Zuo J, Zhu B, Fu D, Zhu Y, Ma Y, Chi L, Ju Z, Wang Y, Zhai B, Luo Y (2012) Sculpting the maturation, softening and ethylene pathway: the influences of microRNAs on tomato fruits. BMC Genom 13:7

Zuriaga E, Blanca J, Nuez F (2009) Classification and phylogenetic relationships in *Solanum* section *Lycopersicon* based on AFLP and two nuclear gene sequences. Genet Resour Crop Evol 56:663–678

Chapter 3
The Importance of Genetic and Epigenetic Research in the *Brassica* Vegetables in the Face of Climate Change

Honghao Lv, Naomi Miyaji, Kenji Osabe, Ayasha Akter, Hasan Mehraj, Daniel J. Shea and Ryo Fujimoto

Abstract The genus *Brassica* includes many economically important crops providing nutrition as well as health-promoting substances. Most cultivars of the *Brassica* vegetables are F_1 hybrids, and breeding system was successfully established by effectively applying the phenomenon of heterosis or self-incompatibility. However, their production is constantly threatened by abiotic and biotic stresses such as the increasing numbers of races and isolates of pathogens, inappropriate cropping systems, and changing climate. Traditional methods of control are often costly and environmentally damaging, while the ideal way is to mine and use the abiotic or biotic resistance from the crop hosts. Fortunately, genomics and molecular genetics enables the rapid discover and application of plant breeding to improve adaptation to environmental conditions and abiotic or biotic resistance. Herein, we have summarized the important characteristics for breeding of the *Brassica* vegetables, including the trials for understanding the molecular mechanisms with genetic and epigenetic approaches. Some future perspectives are also given concerning how to efficiently use these genes and overcome global climate change.

H. Lv (✉)
Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, 12 Zhongguancun South Street, Beijing 100081, China
e-mail: lvhonghao@caas.cn

N. Miyaji · A. Akter · H. Mehraj · R. Fujimoto (✉)
Graduate School of Agricultural Science, Kobe University, 1-1 Rokkodai, Nada-ku, Kobe 657-8501, Japan
e-mail: leo@people.kobe-u.ac.jp

K. Osabe
Plant Epigenetics Unit, Okinawa Institute of Science and Technology Graduate University, Onna-son, Okinawa 904-0495, Japan

A. Akter
Faculty of Agriculture, Bangladesh Agricultural University, Mymensingh, Bangladesh

D. J. Shea
Graduate School of Science and Technology, Niigata University, Ikarashi-ninocho, Niigata 950-2181, Japan

C. Kole (ed.), *Genomic Designing of Climate-Smart Vegetable Crops*,
https://doi.org/10.1007/978-3-319-97415-6_3

Keywords Brassica · Epigenetics · Heterosis · Self-incompatibility · Vernalization · Disease resistance · Abiotic stress

3.1 Introduction

Brassicaceae is a diverse family of angiosperms containing 338 genera and 3,709 species, including the model plant *Arabidopsis thaliana* (Warwick et al. 2006). Three diploid species, *Brassica rapa* L. (AA, $2n = 20$), *Brassica nigra* L. (BB, $2n = 16$), and *Brassica oleracea* L. (CC, $2n = 18$), and three allotetraploid species, *Brassica juncea* L. (AABB, $2n = 36$), *Brassica napus* L. (AACC, $2n = 38$), and *Brassica carinata* L. (BBCC, $2n = 34$), are all involved in the genus *Brassica*, and the relationships of the genome of these six species are known as the triangle of U (Fig. 3.1) (U 1935).

B. rapa and *B. oleracea* show extreme morphological divergence (termed morphotype), which is due to selection by the plant breeders. With this effort, *B. rapa* comprises commercially important vegetable crops consumed worldwide such as leafy vegetables including Chinese cabbage (var. *pekinensis*), pak choi (var. *chinensis*), and komatsuna (var. *perviridis*), root vegetables including turnip (var. *rapa*), and oilseed (var. *oleifera*) (Fig. 3.2) (Cheng et al. 2014, 2016). The heading vegetable, Chinese cabbage, forms a head with large pale green-colored leaves and wide white midribs and is an important vegetable in Asia. The non-heading vegetables, pak choi and komatsuna, are also important vegetables in Asia. Turnip develops enlarged hypocotyls, and there are variations of both shape and color. There are morphotypes of oilseed in *B. rapa*, and seeds are used for oil extraction. *B. oleracea* comprises cabbage (var. *capitata*) from which the leafy heads are harvested, broccoli (var. *italica*) for cluster of flower buds, cauliflower (var. *botrytis*) for enlarged mass of the young, terminal inflorescence (described as the curd), and kohlrabi (var. *gongylodes*) for enlarged stems (Fig. 3.2) (Cheng et al. 2014, 2016). *B. napus* comprises the important oilseed crops such as canola or rapeseed (Fig. 3.2).

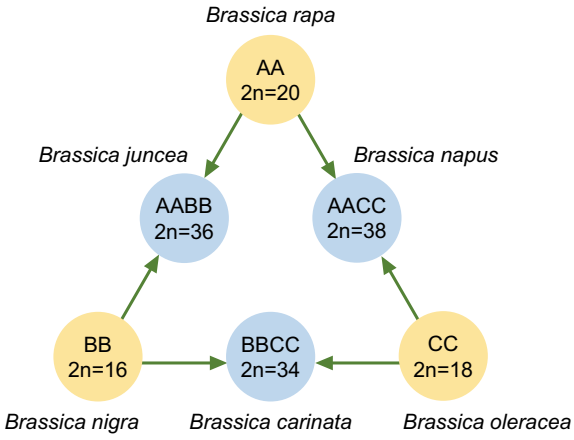

Fig. 3.1 Genetic relationship in the genus *Brassica* known as the triangle of U. Diagram illustrating the genetic relationship between the diploids, *B. rapa* (AA genome), *B. nigra* (BB genome), and *B. oleracea* (CC genome) and the allotetraploids, *B. juncea* (AABB genome), *B. carinata* (BBCC genome), and *B. napus* (AACC genome)

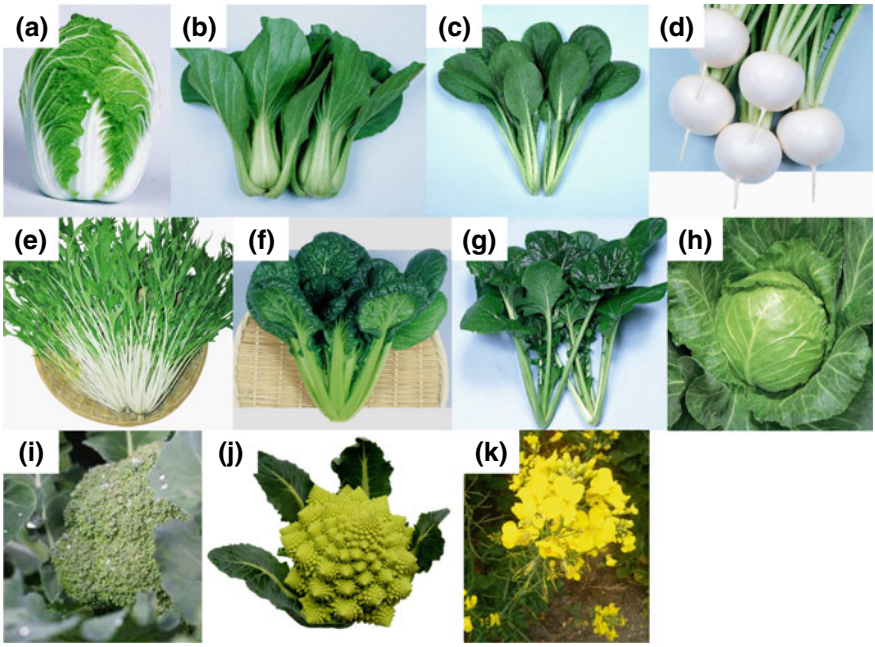

Fig. 3.2 Variations of the *Brassica* vegetables. *B. rapa* vegetables include Chinese cabbage (var. *pekinensis*) (**a**), pak choi (var. *chinensis*) (**b**), komatsuna (var. *perviridis*) (**c**), and turnip (var. *rapa* (**d**), mizuna (var. *japonica*) (**e**), chijimina (var. *narinosa*) (**f**), and sendai-yukina (var. *chinensis*) (**g**). *B. oleracea* vegetables include cabbage (var. *capitata*) (**h**), broccoli (var. *italica*) (**i**), and romanesco (var. *botrytis*) (**j**). *B. napus* crop includes canola (**k**)

Most commercial cultivars of *B. rapa* vegetables such as Chinese cabbage, komatsuna, and turnip or *B. oleracea* such as cabbage, broccoli, and cauliflower, are F_1 hybrids due to their agronomic benefits such as high yield, abiotic stress tolerance, disease resistance, and uniform phenotype (Fujimoto et al. 2018). Hybrid breeding came from the discovery of heterosis (or hybrid vigor), which is defined as the superior performance of hybrid plants over the parents (Crow 1998). In *B. napus*, F_1 hybrid production systems were introduced to replace open-pollinated cultivars leading to increased production. When breeding F_1 hybrid cultivars, breeders developed pure elite lines (inbred lines) as parents for hybrid production. About five to seven generations of selfing and selection based on traits concerned with the breeding objective such as disease resistance are required for developing inbred lines as parental candidates. The level of heterosis of crosses of all possible combinations of the inbred lines is used to identify suitable parents for F_1 hybrid generation. Self-incompatibility or cytoplasmic male sterility is successfully used for the production of F_1 hybrid seeds in *B. rapa* or *B. oleracea* vegetables to avoid contamination by non-hybrid seeds (Fujimoto and Nishio 2007; Yamagishi and Bhat 2014) (Fig. 3.3). The strength of self-incompatibility and stability of male sterility are important for harvesting highly pure F_1 seeds.

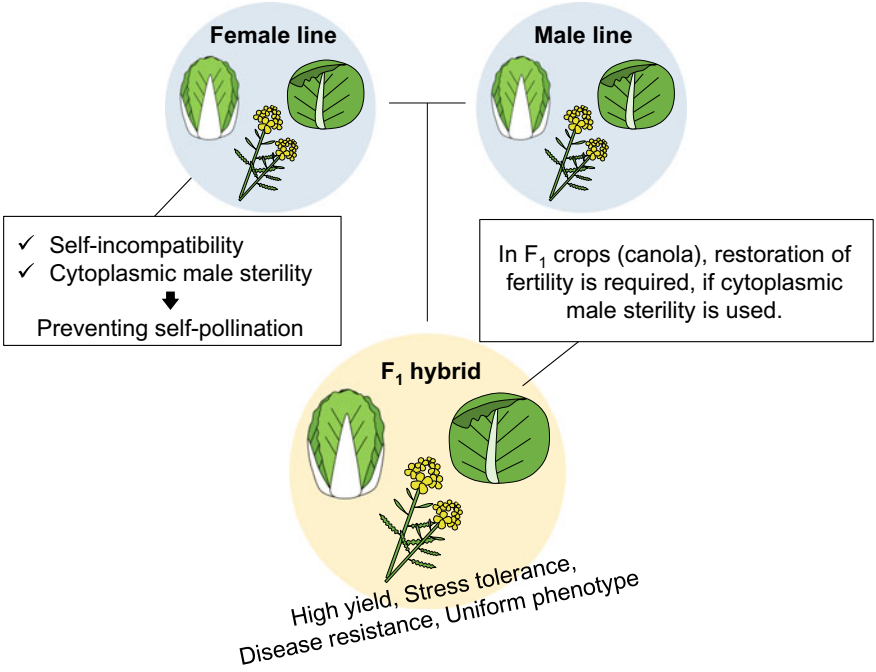

Fig. 3.3 The strategy for production of F_1 hybrid seeds. Breeders develop two elite parental lines to produce F_1 hybrid seeds. To avoid contamination by non-hybrid seeds, self-incompatibility (preventing self-pollination) or cytoplasmic male sterility is successfully used for the production of F_1 hybrid seeds in *B. rapa* or *B. oleracea* vegetables. Fertility needs to be restored to produce seeds of F_1 hybrid crops such as canola

Plants are highly perceptive toward extant environmental conditions. Temperature, water availability and salinity, soil pH and porosity, nutrient availability, and the amount of available photosynthetic active radiation (PAR) in a given geographic region have resulted in evolutionary adaptations to ecological niches that help to ensure successful flowering and germination (Franks and Weis 2009). Adaptation to the seasonal temperature variability experienced in more temperate climates has resulted in vernalization requirements for members of the Brassicaceae family (Shea et al. 2018a). This aims to ensure flowering does not occur until after a plant has perceived a prolonged period of cold and/or the shorter days experienced during the winter season, and promotes flowering during the more amenable spring season (Yan et al. 2003; Huijser and Schmid 2011).

Unlike other organisms, plants are sessile and thus incapable of migratory behavior within a generation. Therefore, migration due to climatic changes can require multiple generations and natural adaptations to environmental changes require evolutionary timescales to develop into viable strategies. The rapidity of current environmental change due to anthropogenic climate change is unprecedented in the geological record (Kemp et al. 2015) and presents a challenge to the increasing demands

placed upon agricultural production (Howden et al. 2007; Namazkar et al. 2015) and very clear and present danger to the ecological stability of flora, and by extension, the fauna that rely on them as a resource for both food and habitat (Montoya and Raffaelli 2010). Along with the aforementioned abiotic factors affected by changes to climate, biotic factors such as disease and insects are of concern. For example, the anticipated increase to humidity and soil temperatures in some regions poses an increased danger from some soilborne pathogens (Das et al. 2016) and insects (DeLucia et al. 2012) and threatens both agricultural and wild cultivation (Newbery et al. 2016). With respect to the *Brassica* vegetables, *Fusarium oxysporum* (responsible for Fusarium wilt and root rot in *Brassica*) and *Plasmodiophora brassicae* (commonly known as clubroot) are of particular concern.

B. rapa is the first species within the genus *Brassica* that has been sequenced, and a doubled haploid (DH) line of Chinese cabbage, chiifu-401-42, was used for sequencing (Table 3.1). Genome sequence information in *B. rapa* and *A. thaliana* revealed that many orthologous genes are conserved (Wang et al. 2011). In addition, *B. rapa* genome has undergone a whole-genome triplication (WGT) after speciation between the genus *Brassica* and *Arabidopsis* (Fig. 3.4) (Wang et al. 2011). This WGT results in multiple copies of paralogous genes. Three subgenomes, the least fractioned subgenome (LF) and two more fractionated subgenomes (MF1 and MF2), were found within the *B. rapa* genome (Cheng et al. 2012). Whole-genome sequence of the other diploid species, *B. oleracea* and *B. nigra*, have been determined (Table 3.1) (Liu et al. 2014; Parkin et al. 2014; Yang et al. 2016). Furthermore, more complicated genomes of allotetraploid species, *B. napus* and *B. juncea*, have also been sequenced (Table 3.1) (Chalhoub et al. 2014; Yang et al. 2016). Recently pangenomes, which refers to a full genomic (genic) makeup of a species, and resequence of the other

Table 3.1 Published representative genomes of the genus *Brassica*

Species	Variety	Assembly method	Database/website	Reference
B. rapa (AA)	Chinese cabbage Chiifu	NGS + SMRT	http://brassicadb.org	Wang et al. (2011)
B. oleracea (CC)	Heading cabbage inbred line 02–12	NGS	http://brassicadb.org	Liu et al. (2014)
B. oleracea (CC)	Kale-like DH line TO1000DH	NGS	http://brassica.info	Parkin et al. (2014)
B. nigra (BB)	Cultivar inbred line YZ12151	NGS	GenBank accession: GCA_001682895.1	Yang et al. (2016)
B. napus (AACC)	Oilseed rape cultivar "Darmor-bzh"	NGS	http://plants.ensembl.org/Brassica_napus	Chalhoub et al. (2014)
B. juncea (AABB)	Vegetable-use cultivar T84-66	NGS +SMRT	GenBank accession: GCA_001687265.1	Yang et al. (2016)

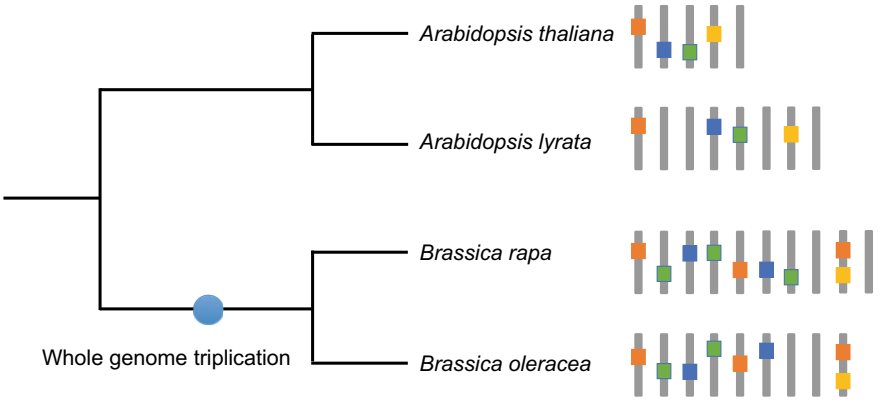

Fig. 3.4 Timing of whole-genome triplication (WGT). There are two or three paralogs by WGT in *B. rapa* and *B. oleracea*, and some paralogs are deleted in *B. rapa* or *B. oleracea* genome after WGT

lines of reference genome were constructed in *Brassica* vegetables using more than one hundred lines within a species (Chen et al. 2015; Golicz et al. 2016; Bayer et al. 2018).

In this chapter, we introduce the important agronomical traits in the genus *Brassica* such as heterosis/hybrid vigor, self-incompatibility, disease resistance (biotic stress), vernalization, and abiotic stress tolerance from the concern of the global climate change.

3.2 What Is Epigenetics

Variation in DNA sequence can cause diverse gene expression changes that influences quantitative phenotypic variation such as morphotypes in the *Brassica* vegetables, which is an important factor determining plant value (Cheng et al. 2016). Gene expression regulatory networks are comprised of *cis*- and *trans*-acting factors, and differences in gene expression are attributable to genetic variation. In eukaryotes, the genome is compacted into chromatin, and the chromatin structure plays an important role in gene expression: gene expression can be controlled by changes in the structure of chromatin that does not involve changes in DNA sequence, and this phenomenon is termed "epigenetic" control (Fujimoto et al. 2012a). Accumulated evidence from researchers has demonstrated that epigenetic change plays an important role in the plant phenotype, and it is also involved in *Brassica* vegetables, such as heterosis, dominance relationship of the pollen determinant of self-incompatibility gene, or vernalization (Fujimoto et al. 2018; Itabashi et al. 2018). DNA methylation and histone modifications are well-known epigenetic modifications that can influence plant phenotype (Fig. 3.5).

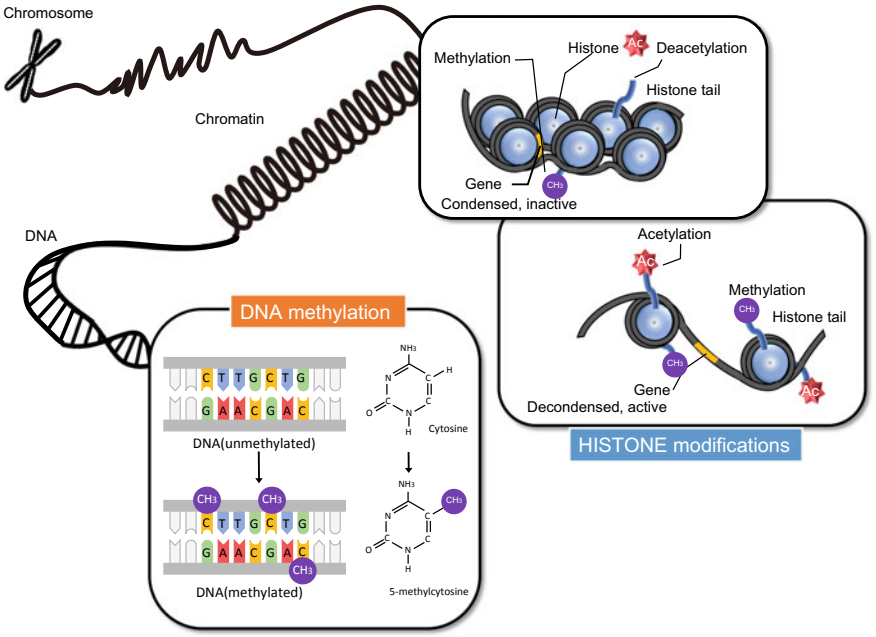

Fig. 3.5 Epigenetic modification. DNA methylation and histone modifications such as methylation or acetylation regulate the structure of chromatin and controls gene expression

3.2.1 DNA Methylation

DNA methylation refers to an addition of a methyl group at the fifth carbon position of a cytosine ring (Fig. 3.5), and in plants, it is observed not only in the symmetric CG context but also in sequence contexts of CHG and CHH (where H is A, C, or T) (Cokus et al. 2008; Lister et al. 2008; Law and Jacobsen 2010; Osabe et al. 2012). DNA methylation is enriched in heterochromatic regions, such as in centromeric and pericentromeric regions, predominantly consisting of transposons (Zhang et al. 2006; Zilberman et al. 2007; Law and Jacobsen 2010). DNA methylation is also observed in euchromatic regions such as gene-coding regions, and it is widely seen in eukaryotes (Feng et al. 2010; Zemach et al. 2010; Vidalis et al. 2016). Genes having DNA methylation at only CG sites of transcribed regions are termed gene body methylation (gbM) (Vidalis et al. 2016).

Spontaneous epimutation is defined as heritable stochastic changes in the methylation states at CG, CHG, and CHH sites, and the rate of epimutation is overwhelmingly higher than the rate of genetic mutations in *A. thaliana* (Becker et al. 2011; Schmitz et al. 2011). Epimutation can sometimes act as the driving force of phenotypic variation (Fujimoto et al. 2012a; Quadrana and Colot 2016). DNA methylation has an important role in the regulation of gene expression, silencing of repeat sequences and transposons, and genome imprinting (Fujimoto et al. 2008a, 2011a; Osabe et al. 2012;

Quadrana and Colot 2016). Most transposons are silenced via DNA methylation, are also immobile to protect genome integrity, and are silenced via DNA methylation (Miura et al. 2001; Singer et al. 2001; Fujimoto et al. 2008b; Tsukahara et al. 2009; Law and Jacobsen 2010; Sasaki et al. 2011).

DNA methylation in CG contexts is largely maintained by METHYLTRANSFERASE I (MET1), and those in CHG contexts are largely maintained by CHROMOMETHYLASE 3 (CMT3)-associated with di-methylation of the 9th lysine of H3 (H3K9me2) (Du et al. 2015; Quadrana and Colot 2016). CHH site methylation is maintained by CMT2 or DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2) (Fig. 3.6) (Zemach et al. 2013; Stroud et al. 2014). The de novo methylation in all sequence contexts is catalyzed by DRM2 and is triggered by 24 nucleotide small interfering RNAs (24 nt-siRNAs) produced by the RNA interference (RNAi) pathway, termed RNA-directed DNA methylation (RdDM). Two plant-specific RNA polymerases, Polymerase IV (Pol IV) and Pol V, together with RNA-dependent RNA polymerase 2 (RDR2), dicer-like 3 (DCL3), and argonaute 4 (AGO4) proteins function in this RNAi pathway (Fig. 3.7) (Matzke and Mosher 2014; Quadrana and Colot 2016).

Genome-wide profiles of epigenetic information define the epigenome, and recent advances in sequencing technology allow us to investigate the epigenome. DNA methylation states at the whole-genome levels have been examined using the methods such as whole-genome bisulfite sequencing (WGBS), methyl-CpG-binding domain sequencing (MBD-seq), epi-restriction-site associated DNA sequencing (EpiRAD-seq), and methylated DNA immunoprecipitation sequencing (MeDIP-seq) (Harris et al. 2010; Laird 2010; Schield et al. 2016). MeDIP-seq is a method to investigate the genome-wide methylation states by high-throughput sequencing enriched for methylated DNA fragments by immunoprecipitation using antibodies raised against methylcytosine (Laird 2010). WGBS directly sequences bisulfite converted DNA,

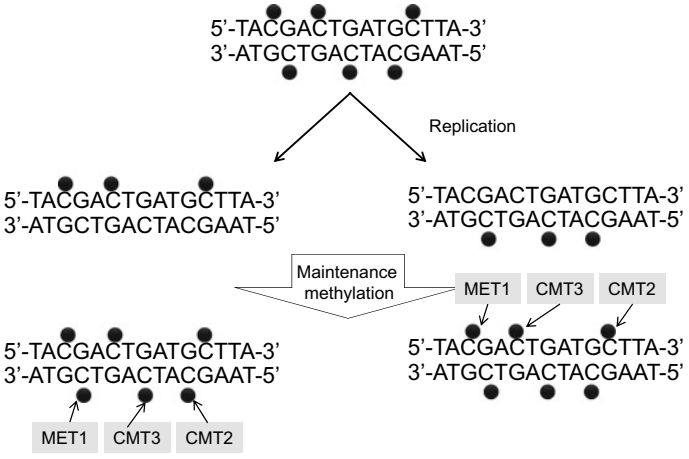

Fig. 3.6 Maintenance of DNA methylation

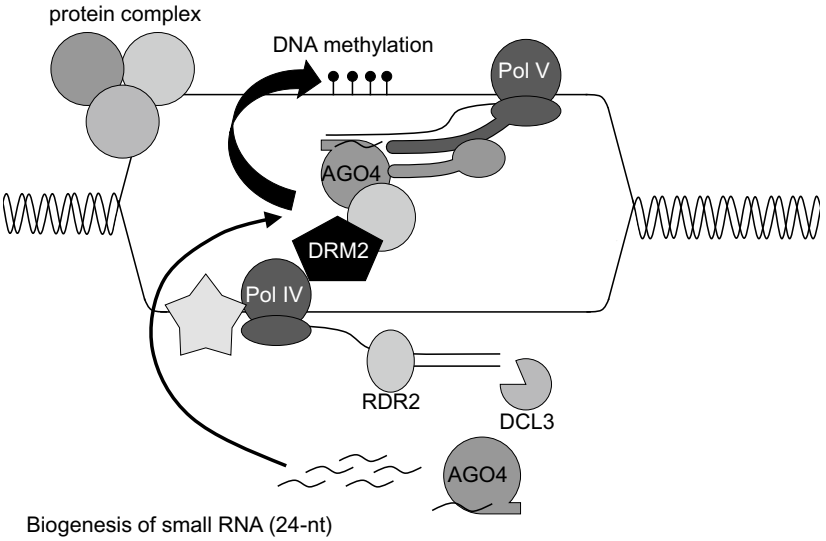

Fig. 3.7 Schematic representation of RNA-directed DNA methylation (RdDM)

and the methylation level at each cytosine position is calculated by dividing the number of methylated cytosines (mC) reads by the total number of reads (Fig. 3.8) (Laird 2010).

Several types of hypomethylated *Brassica* vegetables have been analyzed. Treatment of *B. rapa* with 5-azaC, a cytidine analog that can inhibit DNA methylation, demonstrated male sterility, reduced seed size, and a late flowering phenotype, suggesting a strong relationship between DNA methylation and these traits (Amoah

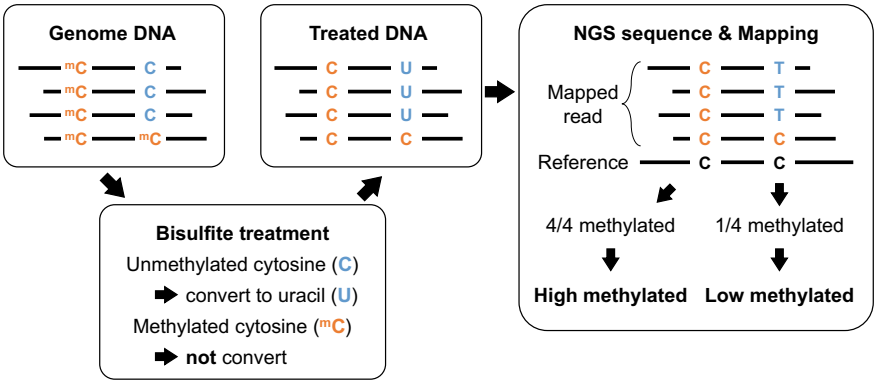

Fig. 3.8 Process of whole-genome bisulfite sequence (WGBS). Unmethylated cytosines (C) are converted to uracil (U) by bisulfite treatments. After bisulfite treatment, whole-genome sequences are determined and the number of C or T is summed for calculation of DNA methylation level (%). WGBS gives the methylated levels of all cytosine sites of the genome

et al. 2012). Hypomethylated transgenic plants in *B. rapa* have been developed by the suppression of *Decrease in DNA methylation 1* (*DDM1*) genes, by RNAi (Fujimoto et al. 2008b). *DDM1* encodes a chromatin-remodeling factor, SWI2/SNF2, and plays an important role in the maintenance of DNA methylation (Vongs et al. 1993; Jeddeloh et al. 1999). *B. rapa ddm1*-RNAi transgenic plants showed reduced levels of DNA methylation and transcriptional reactivation of transposable elements, but they did not show any developmental abnormalities (Fujimoto et al. 2008b; Sasaki et al. 2011). Three mutants, *braA.nrpd1*, *braA.rdr2*, and *braA.nrpe1*, having dysfunction of genes involved in the RdDM pathway have been characterized (Grover et al. 2018). Nuclear RNA polymerase IV, subunit 1 (NRPD1), and Nuclear RNA polymerase V, subunit 1 (NRPE1), are components of the largest subunit of Pol IV and Pol V, respectively. *braA.nrpd1* and *braA.rdr2* reduced the accumulation of 24nt-siRNAs, while *braA.nrpe1* did not show any change. There was no obvious vegetative defect in these three mutants, but silique and seed sizes in all three mutants are smaller than those in wild type (WT). As seed abortion occurs after fertilization, RdDM function is required in maternal sporophytic tissue (Grover et al. 2018).

Whole-genome DNA methylation states have been examined in the *Brassica* vegetables. In *B. rapa*, DNA methylation states have been examined by MeDIP-seq and DNA methylation states were compared between two inbred lines of Chinese cabbage. Most genes having difference of DNA methylation levels between the two lines showed similar gene expression levels, and about 30% of these genes were not expressed (Takahashi et al. 2018a). Using the same lines, tissues, and developmental stages that were harvested independently, WGBS was performed (Takahashi et al. 2018b). Between the MeDIP-seq and WGBS, the WGBS can assess different DNA methylation sequence contexts and was more sensitive (Takahashi et al. 2018a, b). WGBS has also been performed using *B. rapa* by several research groups, and the average methylation levels for CG, CHG, and CHH sites were 52.4%, 31.8%, and 8.3%, respectively (Chen et al. 2015), 37.2%, 17.3%, and 4.4%, respectively (Niederhuth et al. 2016), and 36.5%, 13.4%, and 5.3%, respectively (Takahashi et al. 2018b). This difference could be due to the variation of DNA methylation between lines or tissues. DNA methylation in the upstream and downstream regions of genes is negatively associated with expression levels, especially DNA methylation in the 200-bp upstream and downstream regions (Takahashi et al. 2018b). CHG and CHH methylation in exon or intron regions result in lower expression levels, indicating that CHG and CHH methylation in exon or intron regions are associated with gene silencing (Takahashi et al. 2018b). In contrast, there is no negative association between CG methylation in exons (except for the first exon) and expression levels, and genes having only CG methylation in the exon (gbM) show a moderate expression level, indicating that genes having gbM showed higher expression levels (Takahashi et al. 2018b), which is consistent with gbM genes in other plant species (Vidalis et al. 2016). There is a significant correlation in gbM between orthologous genes in *B. rapa* and *A. thaliana* (Niederhuth et al. 2016; Takahashi et al. 2018b). Significant correlation in gbM between paralogous genes is also found in *B. rapa* (Takahashi et al. 2018b), while the levels of methylation were inversely related to gene expression for each subgenome (DNA methylation: MF1 > MF2 > LF; Gene expression:

LF > MF2 > MF1) (Cheng et al. 2015). The WGBS was also performed in *B. oleracea*, and the average methylation levels for CG, CHG, and CHH sites were 54.9%, 9.4%, and 2.4%, respectively (Parkin et al. 2014). An association between higher expression level and lower DNA methylation level was observed, and gbM related to higher gene expression level. At the subgenome level, lower methylation levels were found in the LF in *B. oleracea* (Parkin et al. 2014).

The 24 nt-siRNA levels are more associated with CHH methylation than CG and CHG methylation in *B. rapa*, suggesting that this CHH methylation was via RdDM (Takahashi et al. 2018b). Furthermore, the average methylation levels for CG, CHG, and CHH sites in the regions overlapping 24 nt-siRNA clusters were quite high even in the non-interspersed repeat regions (IRRs), indicating that 24 nt-siRNA clusters are strongly associated with DNA methylation (Takahashi et al. 2018b).

3.2.2 Histone Modification

Nucleosomes are formed by a histone octamer containing two of each of the core histones H2A, H2B, H3, and H4, and 147 bp of DNA is wrapped around this core. Alteration of chromatin structure, which causes changes in transcription, is regulated by various post-translational modifications of the N-terminal regions of histone proteins, such as methylation or acetylation (Fuchs et al. 2006). Histone lysine residues are able to be mono-, di-, or tri-methylated and each methylation state can be associated with different functions (Fuchs et al. 2006; He et al. 2011). In plants, histone deacetylation, H3K9me2, and H3K27me3 are associated with gene repression, and histone acetylation, H3K4me3, and H3K36me3 are associated with gene activation (Fuchs et al. 2006; He et al. 2011).

Different histone marks can be controlled by different histone lysine methyltransferase and can lead to different effects on gene regulation (Fuchs et al. 2006; Xiao et al. 2016). Histone lysine methyltransferases have a SET domain, which is evolutionally conserved, and SET domains have been identified in *Drosophila melanogaster*; SUPPRESSOR OF VARIEGATION 3-9 (SU(VAR)3-9), enhancer of zeste E(z), trithorax (TRX), and absent, small or homeotic disks 1 (ASH1). In *A. thaliana*, some members of ARABIDOPSIS TRITHORAX (ATX), ARABIDOPSIS TRITHORAX-RELATED (ATXR), and ASH1 HOMOLOG proteins (e.g., ATX1, ATX2, and ASHH2) are involved in H3K4me3 and/or H3K36me3. Histone lysine methyltransferases, KRYPTONITE (KYP)/SU(VAR)3-9 HOMOLOG 4 (SUVH4), SUVH5, and SUVH6, catalyze addition of H3K9me2 (Du et al. 2015). H3K27me3 addition is catalyzed by POLYCOMB REPRESSIVE COMPLEX 2 (PRC2), which is composed of a subset of the Polycomb group (PcG) proteins (Zheng and Chen 2011).

Genome-wide profiles of histone modification are determined by a combination of chromatin immunoprecipitation (ChIP) and genomic tiling arrays (ChIP on chip) or ChIP and high-throughput sequencing (ChIP-seq) (Fig. 3.9), especially to detect methylation and acetylation of lysine residues on histone H3 because histone H3

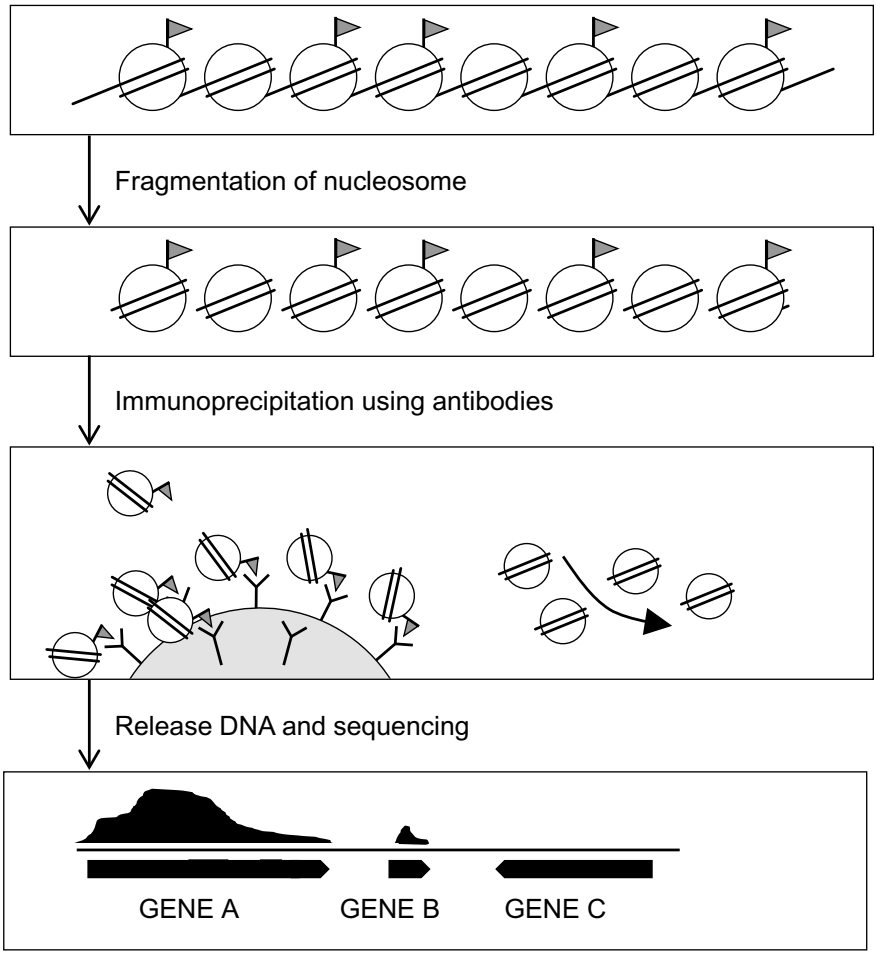

Fig. 3.9 A schematic diagram representing the workflow of chromatin immunoprecipitation sequencing (ChIP-seq). DNA-bound histones are subjected for analyses and antibody against a specific histone modification is used for immunoprecipitation. ChIP DNA is purified and sequenced. The number of mapped reads to reference genome determines the level of histone modification

undergoes the most extensive modification (Xiao et al. 2016). Using these technologies, the genome-wide distribution patterns of histone modifications such as H3K4me3, H3K9me2, H3K27me3, and H3K36me3 have been examined in some plants (Turck et al. 2007; Zhang et al. 2007, 2009; Bernatavichute et al. 2008; Oh et al. 2008; He et al. 2010; Roudier et al. 2011; Makarevitch et al. 2013).

Information about histone modifications is limited in the *Brassica* vegetables. However, positive and negative control primer sets for H3K4me3, H3K9me2, H3K27me3, and H3K36me3 were developed in *B. rapa* (Kawanabe et al. 2016a), and

these primer sets will be helpful for future ChIP analyses in *B. rapa*. Several suggestions were obtained during the process of making these primer sets. (1) H3K4me3 and H3K36me3 are enriched in transcriptionally active genes in *B. rapa*. (2) H3K9me2 is associated with TEs. (3) H3K27me3-targeted genes are conserved between *A. thaliana* and *B. rapa*. However, this has not been confirmed at the whole-genome level except for H3K9me2; a high resolution of the H3K9me2 states was examined in *B. rapa* (Takahashi et al. 2018b). From this ChIP-seq data, H3K9me2 tends to be overrepresented in TEs, but this overrepresentation is lower than DNA methylation and shows a more moderate association with TEs relative to DNA methylation, in *B. rapa*. The average expression level of genes having H3K9me2 in the exon and intron regions are lower than average of total genes. In addition, the level of H3K9me2 associates with DNA methylation levels but not with 24nt-siRNA levels (Takahashi et al. 2018b).

3.3 Heterosis or Hybrid Vigor

Heterosis or hybrid vigor is a phenomenon where hybrid progeny has superior performance compared to their parental inbred lines. The term "heterosis" was replaced to the more cumbersome word "heterozygosis", which did not express the superior performance of the hybrids (Shull 1948). Heterosis is observed in the agronomically important traits such as biomass, yield, and abiotic and biotic stress tolerance. Breeding of F_1 hybrid cultivars based on heterosis is used in many *Brassica* vegetables as well as many other crops (Fig. 3.10).

Historically, F_1 hybrid cultivars were successfully introduced in maize production from 1940s (Crow 1998; Duvick 2001), and interpretation of genetic basis of heterosis began in the 1990s. The famous models such as dominance, overdominance, and epistasis have been suggested to explain the increased biomass and yield (Fig. 3.11) (Schnable and Springer 2013; Fujimoto et al. 2018). These hypotheses have been fundamental to heterosis research, but it is not clear if any one model can explain the molecular mechanism of heterosis. Quantitative trait locus (QTL) analysis is one of the popular approaches for elucidating the genetic bases of agriculturally important traits (Fig. 3.12). QTL analysis has been performed in maize, rice, sorghum, tomato, rapeseed, and cotton in attempts to understand the genetic basis of heterosis (Lippman and Zamir 2007). Most heterosis QTL studies focus on yield-related traits in biparental populations (Lippman and Zamir 2007). Other researchers tried to identify a QTL for general or specific combining ability in hybrids using multiparental populations (Giraud et al. 2017; Zhen et al. 2017). Single-nucleotide polymorphism (SNP) data of large populations have enabled comparisons of genetic architecture in a number of lines. In addition, genome-wide association studies (GWAS) using large SNP data have been incorporated into a genetic approach for heterosis (Fig. 3.13) (Yang et al. 2017a). Recent molecular analyses of transcriptomes, proteomes, and metabolomes, together with reference to the epigenome of the parents and hybrids, have begun to uncover some new facts about the generation of heterosis (Groszmann

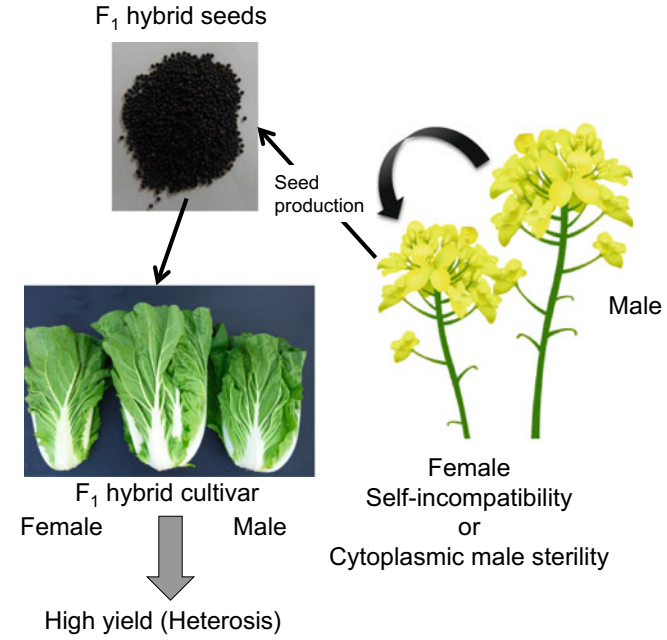

Fig. 3.10 A strategy of F_1 hybrid seed production system in *Brassica* vegetables. Breeders prepare the female and male lines and harvest the F_1 hybrid seeds using self-incompatibility or cytoplasmic male sterility. F_1 hybrid seeds are commercially sold and they have advantage such as high yield due to heterosis

et al. 2011, 2013; Baranwal et al. 2012; Schnable and Springer 2013; Fujimoto et al. 2018; Miyaji and Fujimoto 2018).

In the dominance model, dominant alleles (A and B) suppress or complement the recessive alleles (a and b). In the overdominance model, heterozygosity (B/B') at the key locus contributes to heterosis leading to superior performance. In the epistasis model, nonallelic genes (A and B) inherited from the parental lines interacts and contributes to heterosis.

3.3.1 Relationship Between Genetic Distance and Heterosis

For the crossability test for candidate of parental lines, all possible combinations of the inbred lines are used to identify suitable parents for F_1 hybrid generation. This is expensive, time-consuming, and labor-intensive. Thus, an efficient method for predicting hybrid performance in the parental generations is desired. One of the possible methods candidates the genetic distance between parental lines because it is believed that there is positive correlation between genetic distance and heterosis; crosses between more genetically divergent parental lines lead to greater heterosis

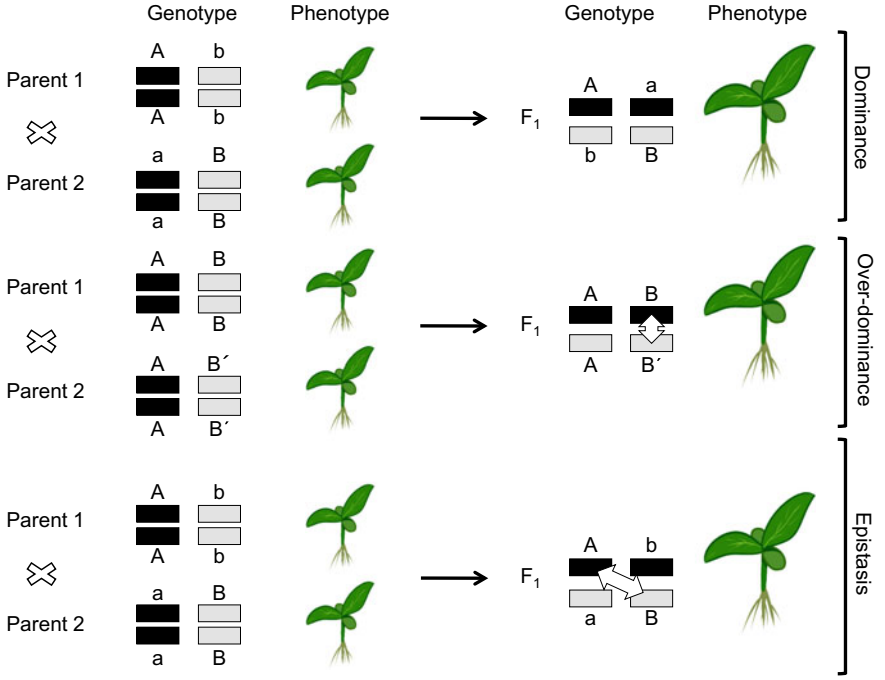

Fig. 3.11 Three hypotheses to explain the genetic mechanism of heterosis

in maize (Moll et al. 1965). However, positive correlation is not always observed between genetic distance and heterosis in plants (Barth et al. 2003; Girke et al. 2012; Yang et al. 2017a).

There are various types of DNA markers used for the analysis and identification of varietal difference in agricultural cultivars. These markers include cleaved amplified polymorphic sequences (CAPS)/restriction fragment length polymorphism (RFLP), amplified fragment length polymorphisms (AFLP), randomly amplified polymorphic DNA (RAPD), simple sequence repeats (SSRs), SNPs, and insertion/deletion polymorphism (InDel) markers (Fig. 3.14). SSR markers have been widely used because of high polymorphism, reproducibility, codominant inheritance, and genome-wide coverage. In addition, SSR markers require only small amounts of DNA for PCR and can be used for high-throughput analysis. SSR markers have been widely used for detecting genetic diversity and making genetic linkage maps, and many SSR markers are available for the genus *Brassica* (Suwabe et al. 2002, 2006; Lowe et al. 2004; Hatakeyama et al. 2010; Pino Del Carpio et al. 2011; Ramchiary et al. 2011; Guo et al. 2014). Sequencing technology enables us to identify SNPs easily, and SNPs are widespread in the *B. rapa* genome (Rafalski 2002; Metzker 2010). SNPs detected by RNA-sequencing (RNA-seq) in coding regions are used for developing gene-based markers (Fig. 3.15) (Paritosh et al. 2013). Restriction-site associated DNA sequencing (RAD-seq), where the flanking region is sequenced from a specific restriction site,

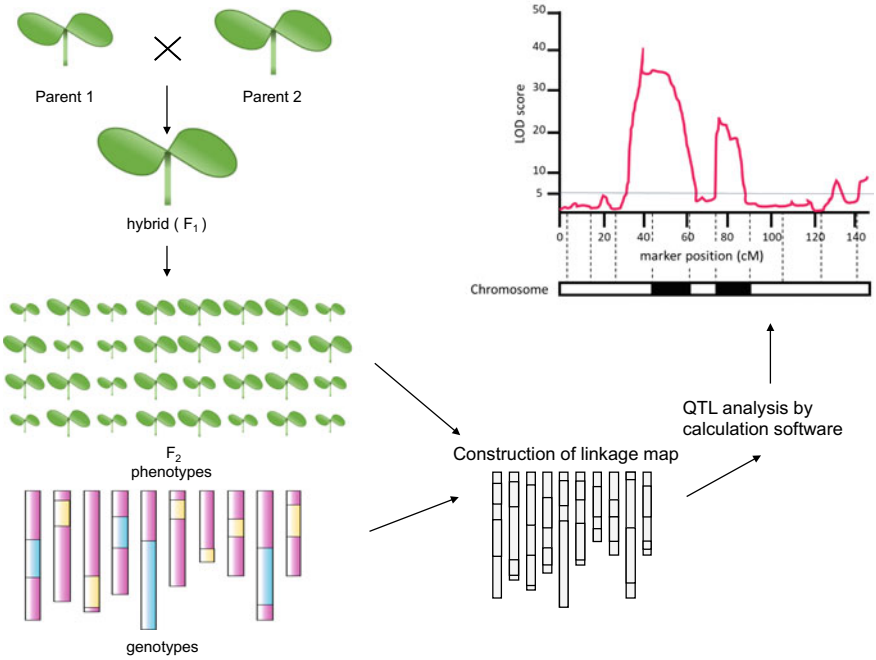

Fig. 3.12 The process of quantitative trait locus (QTL) analysis. The F_2-segregated populations derived from F_1 hybrid are produced. Using phenotype values (plant height, fresh weight, or leaf size, etc.) and genetic information determined by DNA markers, QTL analysis is performed. The genetically linked region with phenotype values is identified as a highest likelihood ratio (LOD) score. The horizontal line in QTL graph shows the significance threshold; therefore, the region between two flanking markers with the LOD peaks above the line is considered as the trait-related locus

is useful for developing DNA markers and high-throughput genotyping (Fig. 3.16) (Baird et al. 2008).

Using 32 F_1 hybrids of Chinese cabbage, genetic distance between parental lines and heterosis levels at three developmental stages was examined. For calculation of genetic distance, three types of DNA markers, SSR (multiallelic markers), CAPS (biallelic markers in exon regions based on SNP information of RNA-seq), and RAD-seq (biallelic markers on SNPs), were used because there is a concern of the ascertainment bias of DNA marker types (Kawamura et al. 2016). The genetic distance measured using the three types of DNA markers showed a high correlation. Of three developmental stages, cotyledon area at 6 days after sowing (DAS), leaf length x width of largest leaf at 21 DAS, and harvested biomass were examined (Fig. 3.17) (Kawamura et al. 2016). The intensity of heterosis is described by means of two indices, the mid-parent heterosis (MPH), and the best-parent heterosis (BPH). MPH is the performance of a hybrid relative to the mean value of its parental lines, whereas BPH is the performance of hybrids relative to the parent having the best value for the trait (Fig. 3.18) (Springer and Stupar 2007). The MPH and BPH were

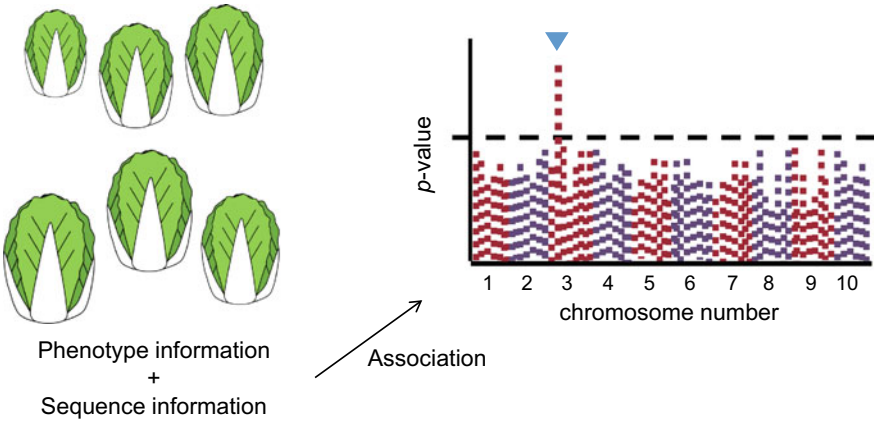

Fig. 3.13 A schematic representation of genome-wide association study (GWAS). GWAS can find associations between DNA mutations and a certain phenotype (plant size in this figure). Manhattan plot makes it easier to visually locate the associations between SNPs and phenotypes. This enables researchers to estimate that the target gene is close to the DNA variants

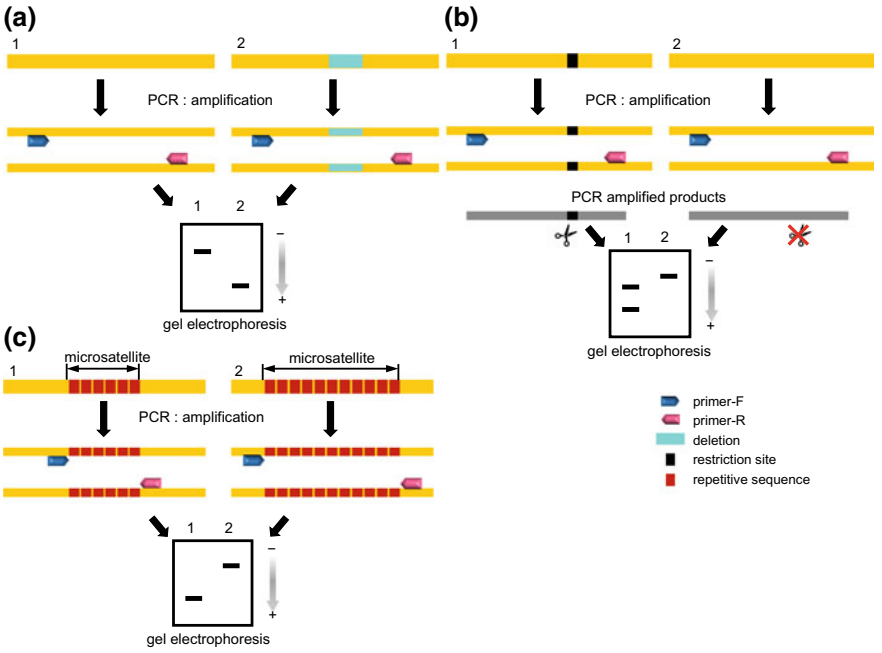

Fig. 3.14 Types of DNA markers. **a** InDel marker can detect the difference of the length of the PCR product when there is an insert/deletion. **b** CAPS marker (RFLP marker). PCR products are digested with a restriction enzyme, and the variation in the recognition site results in different number of bands. **c** SSR marker detects the variation in the number of repeating units of 2–6 base pairs of DNA. After PCR, the fragments can be separated by gel electrophoresis

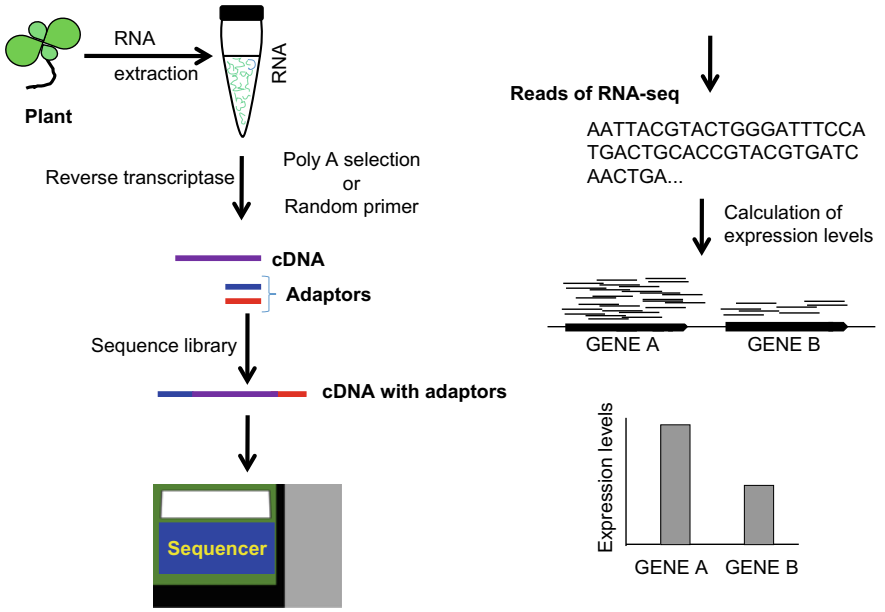

Fig. 3.15 An illustration of the RNA-sequencing (RNA-seq). For making libraries for RNA-seq, the first-strand cDNA is synthesized by random hexamer primers using the short fragmented mRNA, which was purified by beads containing oligo (dT) from the total RNA. The second-strand cDNA is synthesized and sequenced on the high-throughput sequencer. Expression level in each gene is calculated by sequence read number

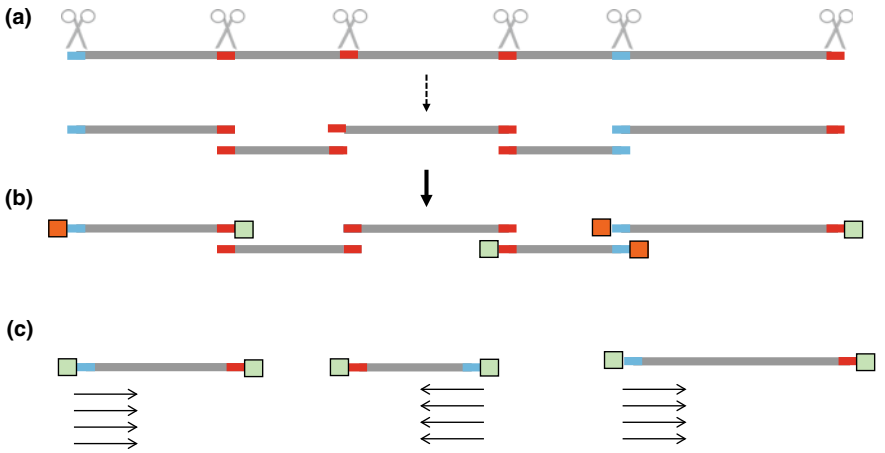

Fig. 3.16 The process of restriction-site associated DNA sequencing (RAD-seq). **a** Genomic DNA is digested with two different restriction enzymes. **b** Adapters (shown as green or orange square) are attached with the ends of restriction enzyme site. **c** Only fragments attached with the different adapters on either end are sequenced, and SNPs are detected from sequence information. RAD-seq targets a subset of the genome, thus providing advantages over whole-genome sequencing including low-cost discovery and genotyping and sequencing of greater numbers of samples

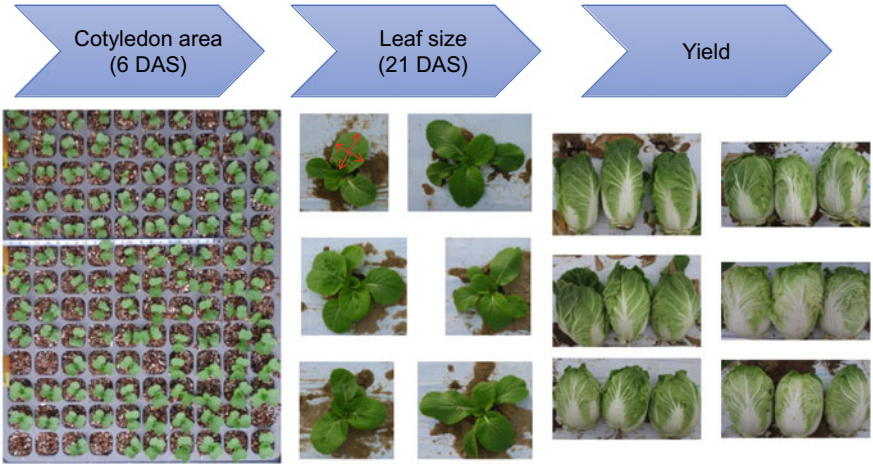

Fig. 3.17 The stages for examination of phenotype in F_1 hybrids and their parental lines in Chinese cabbage

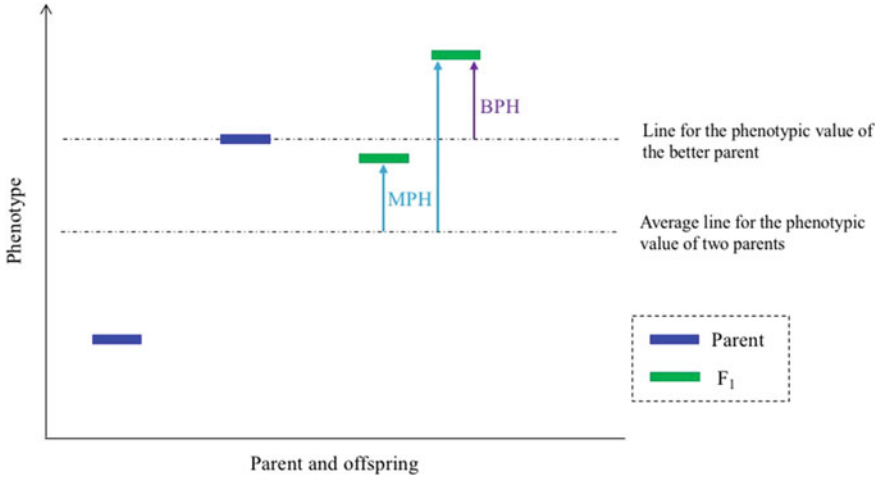

Fig. 3.18 A graphical presentation of mid-parent heterosis (MPH) and best-parent heterosis (BPH). Y-axis indicates the phenotypic values such as biomass in parental lines and their F_1. MPH, heterosis over the mid-parent. BPH, heterosis over the better parent

calculated by the phenotypic data in 32 F_1 hybrids and their parental lines, and the relationship between MPH or BPH and genetic distance of the parental lines was examined. Correlations were not observed between genetic distance and MPH or BPH of the parameter examined (Fig. 3.19) (Kawamura et al. 2016), indicating that the hybrid performance in Chinese cabbage cannot be predicted from the genetic distance of parental lines.

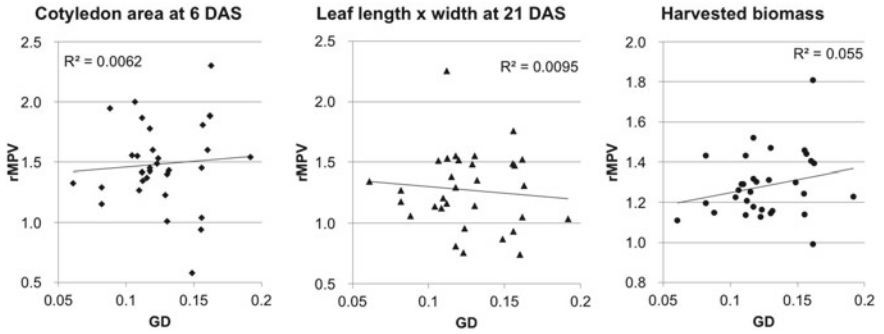

Fig. 3.19 The relationship between genetic distance (GD) and mid-parent heterosis (relative ratio of plant size or biomass between F_1 and mid-parent values (rMPV)) in Chinese cabbage

3.3.2 Early Developmental and Yield Heterosis

The level of heterosis is trait-dependent, and heterosis in yield-related traits is important for F_1 hybrid cultivars (Springer and Stupar 2007; Flint-Garcia et al. 2009; Shi et al. 2011). In the commercial cultivar of Chinese cabbage, "W39", a heterosis phenotype is seen at 4 DAS with hybrids having increased cotyledon size, while there is no difference in cotyledon size at 2 DAS between F_1 hybrids and best parent. The cell number per unit area of the cotyledon was greater for the female parent than the male parent or the hybrid (Fig. 3.20). In the first and second leaves of this F_1 hybrids, leaf size in F_1 hybrids was larger than that in best-parent, and the larger leaf size is associated with increased size and number of the photosynthetic palisade mesophyll cells (Fig. 3.20). Growth speed evaluated by counting leaf number in F_1 hybrids was not faster than parental lines (Saeki et al. 2016). Similar results were observed in the F_1 hybrids of the model plant *A. thaliana* and developed cotyledon with an increased

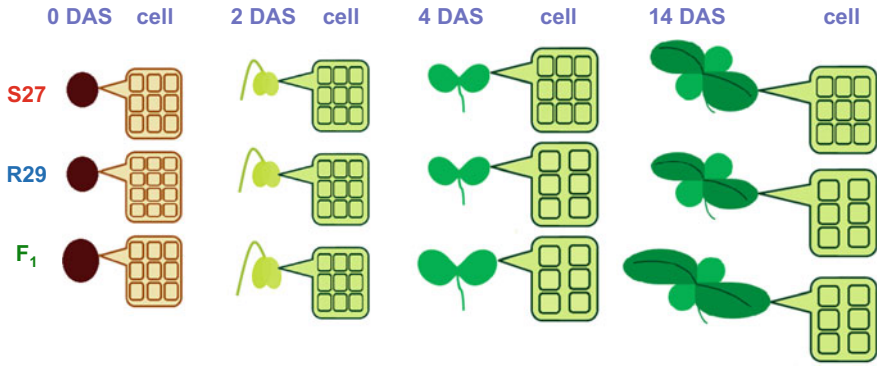

Fig. 3.20 Schematic representation of the size of seed, cotyledon, or leaf and cell size in F_1 hybrid and its parental lines, S27 (female line) and R29 (male line)

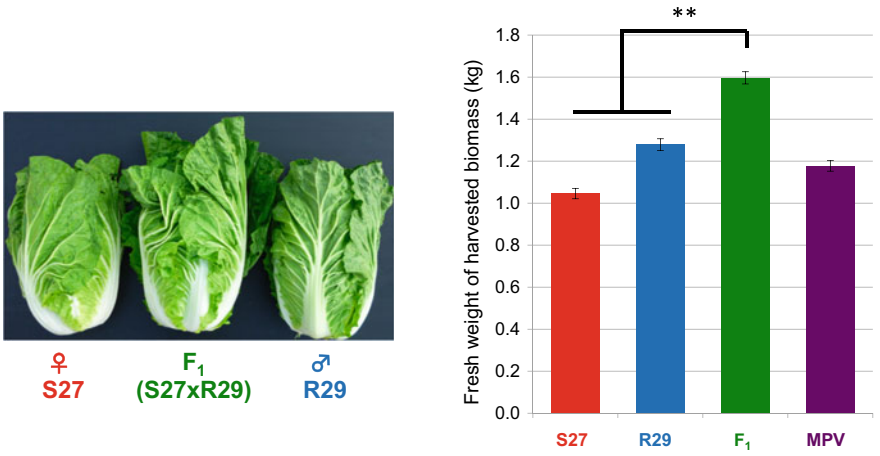

Fig. 3.21 Greater yield in commercial F_1 hybrid

size from a few days after sowing and greater leaf size in the first and second leaves (Fujimoto et al. 2012b; Meyer et al. 2012; Groszmann et al. 2014). Yield heterosis (25% greater than the better parent) was observed in "W39" of Chinese cabbage (Fig. 3.21). The prediction of yield heterosis from the early developmental stages could be useful to save time and labor because commercial F_1 hybrid of Chinese cabbage showed both early developmental and yield heterosis (Saeki et al. 2016). There was a moderate correlation in MPH between leaf size at 21DAS and yield but not in BPH (Kawamura et al. 2016). These results suggest that it is difficult to precisely predict the yield heterosis from the early developmental heterosis, though assessment of heterosis level in early developmental stages may be applied as the first screening of parental combinations of F_1 hybrid cultivars.

Hormone signaling has been suggested to be important in heterotic hybrids of *A. thaliana* (Shen et al. 2012), and a model of hermetic modulation of hybrid vigor (concentration of salicylic acid (SA) in F_1 hybrids is in appropriate range for growth vigor performance) was suggested in *A. thaliana* (Zhang et al. 2016a). However, hormone profiles of 43 derivatives in 2-day cotyledons and 10-day first and second leaves were similar in parental lines and the F_1 hybrid of Chinese cabbage (Saeki et al. 2016).

3.3.3 Transcriptome Analysis in Heterosis

The underlying hypothesis for a transcriptomic approach is that genes whose expression changes in F_1 hybrids may be involved in heterosis. Transcriptome analyses initially used microarray technology and later, RNA-seq have been used to compare parental lines with their F_1 hybrids to identify genes potentially involved in

heterosis (Fig. 3.15). Gene expression levels in F_1 hybrids are classified as additive or nonadditive. Additive gene expression level is defined as the expected changes in gene expression in F_1 hybrids where gene expression levels in F_1 hybrids are equal to the average level of parental gene expression (termed mid-parent value; MPV) (Fig. 3.22). Nonadditive gene expression level is unexpected changes in gene expression in the F_1 hybrids where gene expression levels in F_1 hybrids are either higher or lower than MPV (Fig. 3.22) (Fujimoto et al. 2018). RNA-seq enables us to not only compare the expression level of genes between the F_1 and parental lines but also to examine the parental allelic contributions to gene expression in F_1 hybrids at the whole-genome level (Chodavarapu et al. 2012).

Upregulation of chloroplast-targeted genes occurs in the heterotic intraspecific hybrids of *A. thaliana* and rice, and the heterotic interspecific hybrids of *A. thaliana* and related species (Ni et al. 2009; Song et al. 2010; Fujimoto et al. 2011b, 2012b; Tonosaki et al. 2016). In F_1 hybrids of Chinese cabbage, "W39", gene expression levels of eight chloroplast-targeted genes were examined by quantitative RT-PCR (RT-qPCR). Most genes showed higher expression levels in F_1 hybrids than in parental lines at 2 DAS, though expression level per se is low. At 3DAS, the expression levels of these genes increase in both F_1 hybrids and parental lines, but there was no difference in expression levels between F_1 hybrids and parental lines. From 4 to 6 DAS, there was no difference in expression levels between F_1 hybrids and parental lines. These results indicate that upregulation of chloroplast-targeted genes occurs at a specific developmental stage (Saeki et al. 2016). RNA-seq using 2-day cotyledons in F_1 hybrid and its parental lines of Chinese cabbage showed genes categorized into "Photosynthesis" and "Chloroplast part" tended to be upregulated, suggesting that

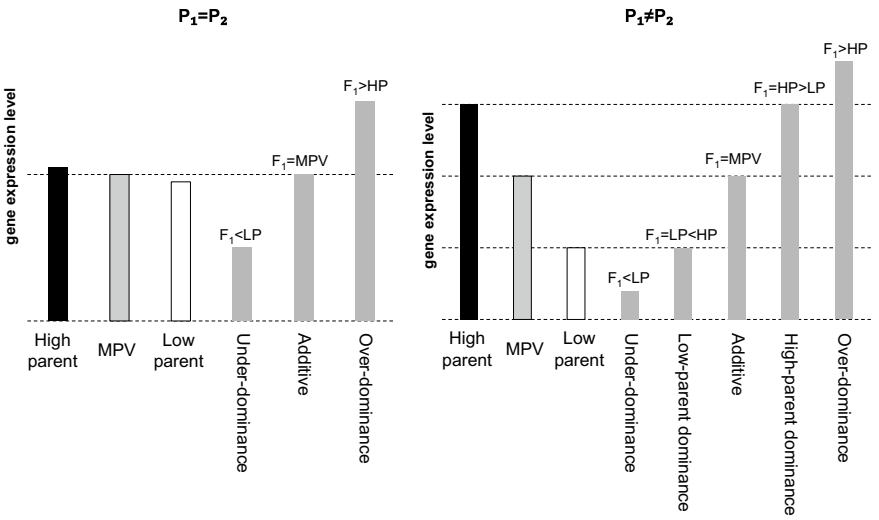

Fig. 3.22 Classification of the mode of gene action in F_1 hybrid (F_1) compared with parental gene expression level (P_1 or P_2). LP; low-parent, HP; high-parent, MPV; mid-parent value

chloroplast-targeted genes are upregulated at the whole-genome level. Stress-related genes tended to be downregulated in F_1 hybrids compared with in parental lines (Saeki et al. 2016).

As RNA-seq enables us to distinguish the parental alleles of transcripts in F_1 hybrids using SNP information, the parental alleles expressed in the F_1 hybrid of Chinese cabbage were examined. Most genes showed that differences in the expression levels between parental lines are maintained in the allelic bias of transcripts in F_1 hybrids (Saeki et al. 2016). Some genes showed allele-specific expression, and these genes tended to be categorized into "Translation" and "Ribosome" (Saeki et al. 2016).

3.3.4 Resequencing and SNP Analysis of the Parental Lines of a Commercial F_1 Hybrid Cultivar

SNP identification through the whole-genome resequencing of cultivar varieties has identified allelic mutations. Comparative variome analysis in a *B. rapa* collection has been reported and identified millions of high-quality SNPs (Cheng et al. 2016). The application of SNP markers has been used to identify seed coat color, hairiness, leaf morphology, and flowering time in *B. rapa* (Rahman et al. 2007; Zhang et al. 2008; Li et al. 2009). The functional loss of genes caused by SNPs and the distribution of high impact SNPs in comparison to the *B. rapa* reference genome sequence is desirable for trait analyses and breeding programs.

SNPs, genome structure, and composition between parental lines of the F_1 hybrid cultivar of Chinese cabbage, "W77", were examined especially in protein-coding genes, by resequencing the genomes of the parental lines (Shea et al. 2018b). Not only moderate impact SNPs, nonsynonymous mutations without changing the framework of amino acid sequence but also high impact variants causing frameshifts, nonsense mutations, or other mutations that could possibly result in the loss of gene function were identified in both parental lines (Shea et al. 2018b). These putative nonfunctional genes that occurred specifically in each parent were distributed throughout the chromosome with high density. Functional markers derived from polymorphisms within genes that affect phenotypic variation are especially valuable in plant breeding, and thus these SNPs leading to nonfunctional genes will be applied to make functional markers that can assist future functional gene studies. If the dominance hypothesis (superior performance of hybrids results in the suppression/complementation of deleterious recessive alleles from one parent by beneficial or superior dominant alleles from the other (Crow 1998; Jones 1917)) applies to heterosis in Chinese cabbage, these putative loss-of-function genes in one parent could be the best candidate genes for heterosis of yield in Chinese cabbage.

Furthermore, the parental line-specific mutations in *Eco*RI sites by genome-wide comparative analysis were identified, and CAPS markers were developed. These CAPS markers can distinguish parental genotypes with codominance using agarose

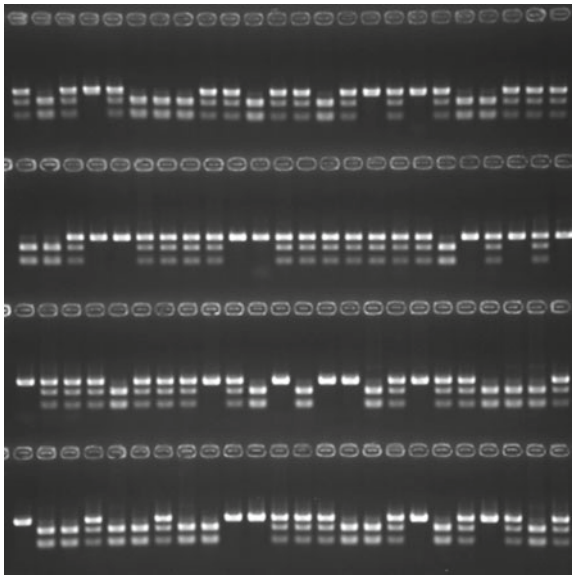

Fig. 3.23 Example of the electrophoresis of the genotyping. 96 F_2 plants derived from F_1 hybrid cultivar "W77" and CAPS marker developed were used. One band is in S11, two bands are in R09, and three bands are in F_1

gel electrophoresis (Fig. 3.23), providing an easy and low-cost method of genotyping, suggesting that they can be applied for genetic analysis such as QTL analysis.

3.3.5 Epigenetic Regulation and Heterosis

Recent study has revealed the possibility of an epigenetic contribution to heterosis. Enhanced growth similar to heterosis was observed in several of the hybrids between WT and specific epigenetic recombinant inbred lines (epiRIL) in *A. thaliana* (Dapp et al. 2015; Lauss et al. 2018). epiRILs differ only in DNA methylation levels, and their genetic backgrounds are almost the same (Johannes et al. 2009; Reinders et al. 2009; Teixeira et al. 2009). These two researches using hybrids between WT and epiRILs suggest that heterosis results in the difference of DNA methylation states between parental lines (Dapp et al. 2015; Lauss et al. 2018). In addition, two groups showed that DDM1 is a major regulator of heterosis in *A. thaliana* (Kawanabe et al. 2016b; Zhang et al. 2016a). The F_1 hybrids having homozygous mutations in *ddm1* had reduced vegetative heterosis (Fig. 3.24). As DDM1 is involved in maintenance of DNA methylation, alterations in DNA methylation affect the level of heterosis (Kawanabe et al. 2016b; Zhang et al. 2016a).

There are few reports studying heterosis from the aspects of epigenetic regulation in *Brassica* vegetables. The hybrid broccoli, which showed larger curds, bigger leaves, and greater roots, was used for transcriptome and methylome analysis (Li et al. 2018b). Methylation-dependent restriction-site associated DNA (MethylRAD) method was used for methylome analysis. The DNA methylation levels were slightly

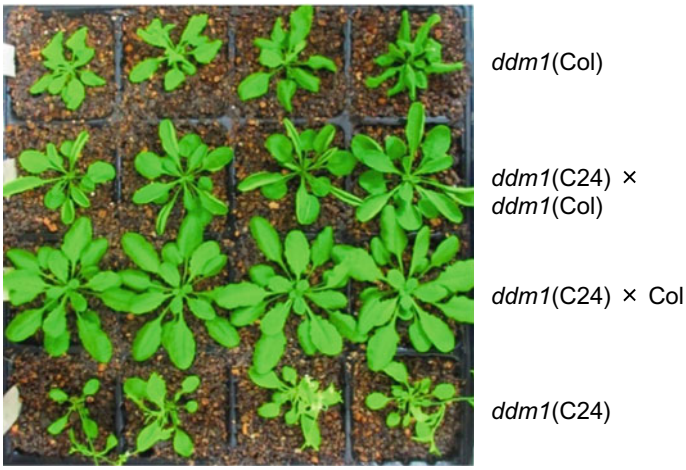

Fig. 3.24 The F_1 hybrid between *ddm1* mutant in Columbia (Col) and *ddm1* mutant in C24 showed reduced level of heterosis. This result indicates that DDM1 plays a role in increasing leaf area of F_1 hybrids

higher in F_1 hybrids than MPV, and most of differentially methylated regions were intergenic. In addition, difference of DNA methylation in genes did not result in their difference of gene expression level (Li et al. 2018b). Although not so large, increased DNA methylation levels were observed in the heterotic hybrids of other plant species (Greaves et al. 2012; Shen et al. 2012, 2017). However, there is little evidence that the difference of DNA methylation between F_1 hybrids and parental lines directly affected differential gene expression between them. As mentioned above, the possible involvement of DNA methylation on heterosis has been proposed (Dapp et al. 2015; Kawanabe et al. 2016b; Zhang et al. 2016a; Lauss et al. 2018; Miyaji and Fujimoto 2018), and change of DNA methylation states in heterotic F_1 hybrids was revealed (He et al. 2010; Chodavarapu et al. 2012; Greaves et al. 2012; Shen et al. 2012, 2017). However, direct evidence is not yet obtained, and further study will be required.

3.3.6 Perspective

The F_1 hybrid cultivars have contributed to increasing crop yields during the last century. However, we still cannot predict the intensity of heterosis before the F_1 hybrids have been produced. This is because breeding of F_1 hybrid cultivars is still laborious, time-consuming, and costly. Most heterosis research has focused on growth vigor or increased yield, but there are a few reports showing the heterosis in biotic or abiotic stress tolerance (Rohde et al. 2004; Miller et al. 2015; Yang et al. 2015). Stable production will be more important for F_1 hybrid cultivars of *Brassica* vegetables by

global climate change, and combining heterosis in different characters such as yield heterosis and stress tolerance heterosis could lead to producing better cultivars.

3.4 Self-incompatibility

Many species in the genus *Brassica* have a self-incompatibility system, which is controlled by a single *S* locus with multiple alleles (Bateman 1955). The determinants of self-recognition specificity in the stigma and the pollen have been identified; the female and male determinants are named *S* receptor kinase (SRK) and *SP11/SCR* (*S*-locus protein 11/*S*-locus cysteine rich) (*SP11* hereafter), respectively (Stein et al. 1991; Schopfer et al. 1999; Suzuki et al. 1999; Takasaki et al. 2000). SRK is a membrane-spanning serine–threonine kinase and has an extracellular domain (*S* domain), a transmembrane domain, and an intracellular domain (kinase domain) (Stein et al. 1991). SP11 is a small cysteine-rich protein (Schopfer et al. 1999; Suzuki et al. 1999). These two determinants interact with each other in an allele-specific manner (Kachroo et al. 2001; Takayama et al. 2001), and the interaction of these two factors induces reactions of self-pollen rejection (Fig. 3.25). Many cultivars of *Brassica* vegetables are F_1 hybrids that are produced using the self-incompatibility

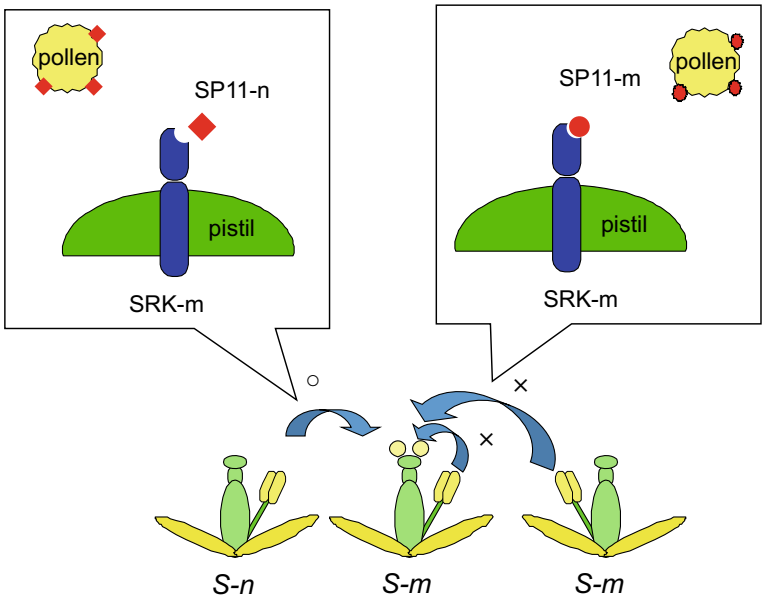

Fig. 3.25 Schematic representation of the self-incompatibility triggered by allele-specific interaction between SRK and SP11. SRK-m and SP11-m can interact with each other and this *S* haplotype-specific interaction leads to self-incompatibility. SRK-m and SP11-n cannot interact with each other, and thus *S-n* is compatible to *S-m*

system. Now, DNA-based methods can examine whether two lines are compatible without performing a crossing test.

3.4.1 Sequence Diversity of Multiple Alleles Located on S Locus

S determinants, *SRK* and *SP11*, are closely linked to each other in the *S* locus, and the alleles of these two genes are transmitted to the progeny together as a set. Therefore, this set of alleles is termed "*S* haplotype" (Fujimoto and Nishio 2007). The first candidate protein identified as the female *S* determinant, *S*-locus glycoprotein (SLG), is also located in the *S* locus and segregates with *SRK* and *SP11*. There is a high degree of sequence similarity between *SLG* and the *S* domain of *SRK* (Stein et al. 1991). About 50 and 30 *S* haplotypes have been identified in *B. oleracea* and *B. rapa*, respectively (Nou et al. 1993; Ockendon 2000).

Nucleotide sequences of *SLG*, *S* domain of *SRK*, and *SP11* of many *S* haplotypes have been determined in *B. rapa* and *B. oleracea*. There are sequence variations in *SLG*, *S* domain of *SRK*, and *SP11* among the *S* haplotypes of *B. rapa* or *B. oleracea* (Kusaba et al. 1997; Sato et al. 2002). Deduced amino acid sequences of *SP11* are more variable than *SRK* or *SLG* in *B. rapa* and *B. oleracea* (Watanabe et al. 2000; Sato et al. 2002). On the basis of nucleotide sequences in these genes, *S* haplotypes are classified into two groups, class-I and class-II (Fujimoto and Nishio 2007). The sequence variations of *S* domain of *SRK* and class-II *SP11* between *S* haplotypes have relatively less nucleotide sequence variation compared with class-I *S* domain of *SRK* and *SP11,* respectively (Shiba et al. 2002), suggesting that class-II *S* haplotype diversification occurred more recently than that of the class-I *S* haplotypes.

3.4.2 Conservation of the Recognition Specificity After Speciation Between B. rapa and B. oleracea

From the sequence information of *SLG*, *S* domain of *SRK*, and *SP11* in *B. rapa* and *B. oleracea,* interspecific pairs of *S* haplotypes, which have a high-sequence similarity of both female and male *S* determinants between species, are identified (Kusaba et al. 1997; Sato et al. 2002, 2003). The same recognition specificity between interspecific pairs has been proved by pollination tests using interspecific hybrids, transgenic plants, and bioassay of recombinant SP11 proteins (Kimura et al. 2002; Sato et al. 2003, 2006), indicating that interspecific pairs between *B. rapa* and *B. oleracea* have the same recognition specificities.

The important regions for the recognition specificities of SRK and SP11 have been investigated by comparing amino acid sequences of SRK and SP11 in interspecific pairs. There were few amino acid substitutions in hypervariable regions (HVRs)

of SRK between interspecific pairs, although the HVRs are highly variable among different *S* haplotypes, suggesting that the HVRs in SRK are important regions for recognition specificities (Sato et al. 2003). In SP11, the important regions for the recognition of the same haplotype of *S* domain of SRK have been identified by domain swapping or alanine-scanning mutagenesis (Chookajorn et al. 2004; Sato et al. 2004).

3.4.3 The Diversification of the Genome Structure of S Locus

The genome structure of the *S* locus has been investigated in some *S* haplotypes of *B. rapa* and *B. oleracea* (Fujimoto et al. 2006a). In the center of the *S* locus of *B. rapa*, gene placement, distance between *SP11*, *SRK*, and *SLG*, and the orientation of these genes are different between *S* haplotypes, while sequence polymorphism in the flanking sequence is lower (Fig. 3.26) (Fujimoto et al. 2006a; Takuno et al. 2007). Recombination between *SRK* and *SP11*, which results in the breakdown of self-incompatibility, seldom occurs, and recombination suppression is considered to be mainly due to the heteromorphism of the *S* locus. Between *BrS-8* and *BrS-46*, which has a highly homologous region in *SLG* and the third to seventh exons of *SRK,* and recombination is detected in a part of *SLG* and part of *SRK* identified by the comparison of the whole-genome sequence of the *S* locus regions between these *S* haplotypes (Fig. 3.27) (Kusaba and Nishio 1999; Takuno et al. 2007). In this case, recombination within the *S* locus was identified, but this recombination did not result in the self-incompatibility recognition, suggesting it was not selected out. Comparison between class-I and class-II *S* haplotypes of *B. rapa* showed that genome structure of the *S* locus of a class-II *S* haplotype is similar to that of class-I *S* haplotypes, but that the order of *SRK* and *SLG* in the class-II *S* haplotype is reversed compared to the class-I *S* haplotypes (Fukai et al. 2003).

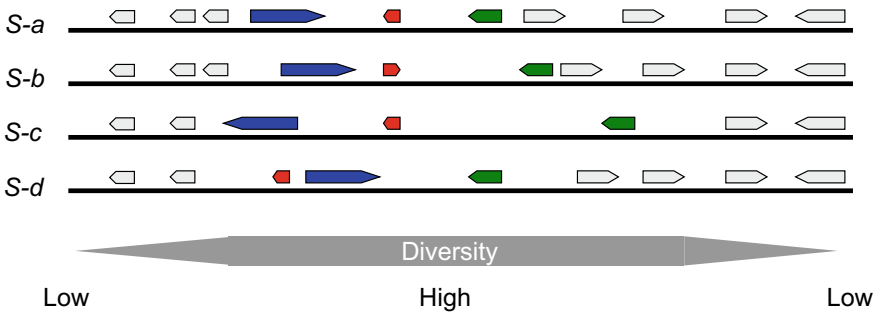

Fig. 3.26 Schematic representation of structural polymorphism of the *S* locus. In the center of *S* locus region covering *SRK* (blue), *SP11* (red), and *SLG* (green) is diverged, while in the flaking region having high sequence homology

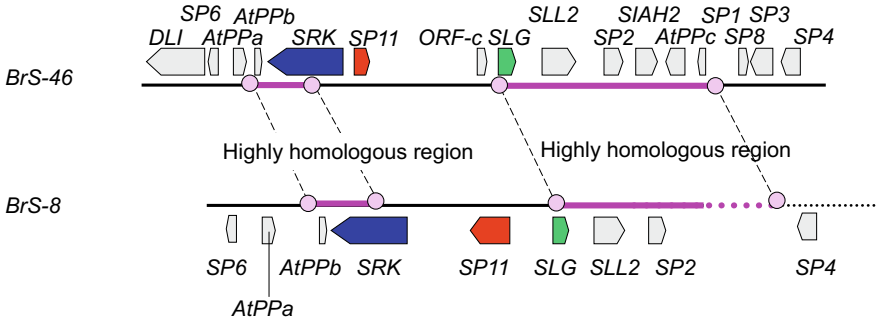

Fig. 3.27 Comparison of the genome structure of *S* locus regions between *BrS-8* and *BrS-46*. Regions having high sequence homology are due to the recombination between these two *S* haplotypes

Interspecific pairs of *S* haplotypes are useful for the comparison of the *S* locus genome structure between species because *S* locus structure diverges within species and the ancestral *S* locus is common between interspecific pairs. Comparison of the structure of the *S* locus in three interspecific pairs demonstrated that the *B. oleracea S* locus is larger than the *B. rapa S* locus and revealed more retrotransposon-like sequences, termed *S-locus retrotransposon families* (*STF*s), in the *S* locus of *B. oleracea* than in that of *B. rapa* (Fujimoto et al. 2006a, 2008c). Most *STF*s are considered to have been inserted after speciation of *B. rapa* and *B. oleracea* (Fig. 3.28) (Fujimoto et al. 2006a, b). This transposable insertion into the *S* locus in *B. oleracea* may not be due to a specific event in the *S* locus because in most of the synthetic regions between *B. rapa* and *B. oleracea*, the region in *B. oleracea* is larger and contains many transposable elements than in *B. rapa* (Liu et al. 2014).

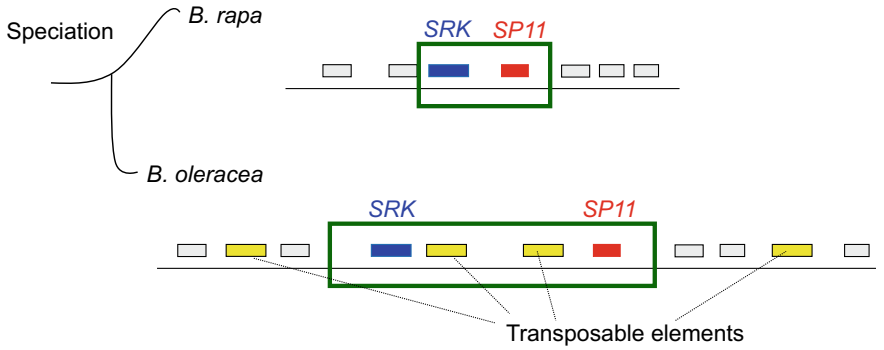

Fig. 3.28 Difference of the *S* locus regions between *B. oleracea* and *B. rapa*. *S* locus region in *B. oleracea* is larger than those in *B. rapa*, and this is due to the insertion of transposable elements in *B. oleracea* after speciation

3.4.4 Self-compatibility Results in the S Determinant Genes But also in the Downstream Genes of S Haplotype-Specific Interactions

Most plants in *B. rapa* and *B. oleracea* are self-incompatible, and there are a few self-compatible lines obtained by spontaneous mutations, suggesting an advantage of self-incompatibility in these species. There is a self-compatible line of Chinese kale, *B. oleracea* var. *alboglabra*. This line has the deletion of both the *S* domain and the transmembrane domain of *SRK* and *SP11* (Nasrallah et al. 1994; Fujimoto et al. 2006b).

"Yellow sarson" is a self-compatible oilseed cultivar (*B. rapa* var. *oleifera*) in India. The self-compatibility of "Yellow sarson" is controlled by two loci, *S* and *M*, and the *M* locus is independent of the *S* locus (Hinata et al. 1983). "Yellow sarson" does not express *SRK* nor *SP11*, which is due to an insertion of a retrotransposon in *SRK* and deletion of the promoter region of *SP11* (Nasrallah et al. 1994; Watanabe et al. 1997; Fujimoto et al. 2006b). *M-locus protein kinase* (*MLPK*) has been isolated as a candidate gene of *M* by map-based cloning (Fig. 3.29). MLPK belongs to a subfamily of receptor-like cytoplasmic kinase (RLCK). *MLPK* of "Yellow sarson" has one amino acid substitution by a single-nucleotide change that leads to the loss of the autophosphorylation activity (Murase et al. 2004). Direct interaction between MLPK and SRK and phosphorylation of MLPK by SRK *in vitro* has been confirmed (Kakita et al. 2007a, b). There are two isoforms of MLPK by alternative transcriptional initiation sites; one localizes to the papillae cell membrane by myristoylation dependency and the other localizes to the plasma membrane by N-terminal hydrophobic region. Each MLPK isoform can complement the *mlpk* mutation (Kakita et al. 2007a). These results suggest that MLPK is involved in the downstream process of the *S*-allele-specific interaction through direct interaction with SRK.

Self-compatible plants from a self-pollinated population of an F_1 hybrid cultivar, "CR-Seiga 65" in Chinese cabbage having heterozygosity of *BrS-46* and *BrS-54* were identified. Pollination tests indicated that this self-compatibility is linked to *BrS-54* and that the recognition function of the stigma is lost. The SRK allele of this self-compatible plant, named BrSRK-54f, is normally transcribed and translated, but gene conversion from *SLG* to *SRK* occurred resulting in the loss of the recognition specificity of BrSRK-54 (Fig. 3.30) (Fujimoto et al. 2006c).

3.4.5 Dominance Relationship of S Haplotypes

Because self-incompatibility in *Brassica* vegetables is sporophytically controlled, there are dominance relationships of *S* haplotypes in the stigma and pollen (Thompson and Taylor 1966). Codominance is common and observed more frequently in the stigma than that in the pollen. The dominance relationships are different between the stigma and the pollen, and the dominance order of *S* haplotypes is nonlinear except

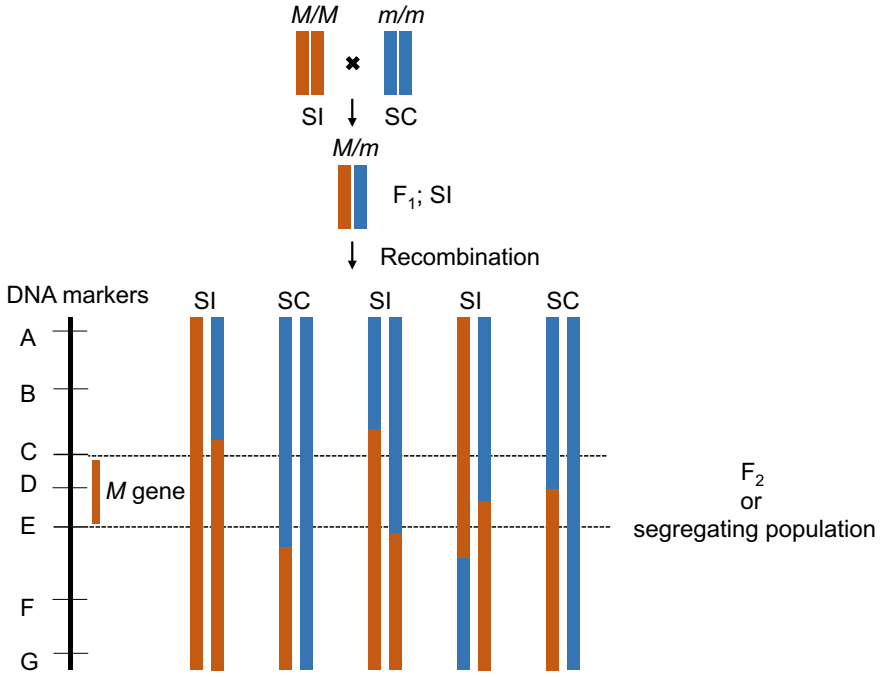

Fig. 3.29 Schematic representation of map-based cloning. Map-based cloning is a method to identify the location of a candidate gene using the genetic and phenotypic information. In this case, genotype was determined using DNA markers in segregating population. Phenotype, SI (self-incompatibility) or SC (self-compatibility), is also determined by pollination test. From genotype and phenotype data, the location of *M* gene is identified in the region between marker-C and marker-E

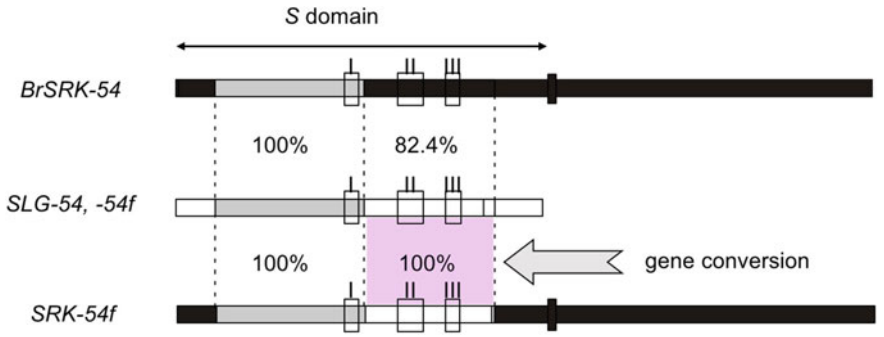

Fig. 3.30 Evidence of the gene conversion between *SLG* and *S* domain of *SRK* genes within the same *S* haplotype. This gene conversion results in the loss of recognition specificity of SRK

for dominance relationship between class-II *S* haplotypes in the pollen (Thompson and Taylor 1966; Hatakeyama et al. 1998; Kakizaki et al. 2003; Yasuda et al. 2016).

The dominance relationship in the stigma is considered to be determined by the SRK protein itself; two models, competition of SRK-mediated signaling pathway and post-transcriptional modification of SRK, are proposed (Hatakeyama et al. 2001). The former model suggests the importance of the kinase domain in determining the dominance relationships of the SRK alleles; however, it is not clear which of the *S* domain or the kinase domain is important for determining the dominance relationship of SRK.

In pollen, class-I *S* haplotypes are dominant over class-II *S* haplotypes in the class-I/class-II *S* heterozygote plants of pollen (Nasrallah et al. 1991). In class-I/class-II *S* heterozygotes, expression of class-II *SP11* is suppressed and the promoter region of class-II *SP11* is DNA methylated (Fig. 3.31) (Shiba et al. 2002, 2006; Tarutani et al. 2010). The class-I *S* haplotypes have the *SP11*-methylation-inducing region (SMI) located in the *S* locus, and its sequence has homology to the promoter region of class-II *S* haplotypes (Fig. 3.31). The 24nt-small RNAs, *Smi*, are expressed from SMI during early stages of anther development, and these small RNAs can trigger de novo DNA methylation of the promoter region of class-II *SP11* (Fig. 3.31). This indicates that class-I derived *Smi* induces silencing of the recessive *SP11* allele by *trans*-acting de novo DNA methylation in the class-I/class-II *S* heterozygote plants (Tarutani et al. 2010). Between the class-II *S* haplotypes, there is a linear dominance order in pollen (*BrS-44* > *BrS-60* > *BrS-40* > *BrS-29*), and DNA methylation is

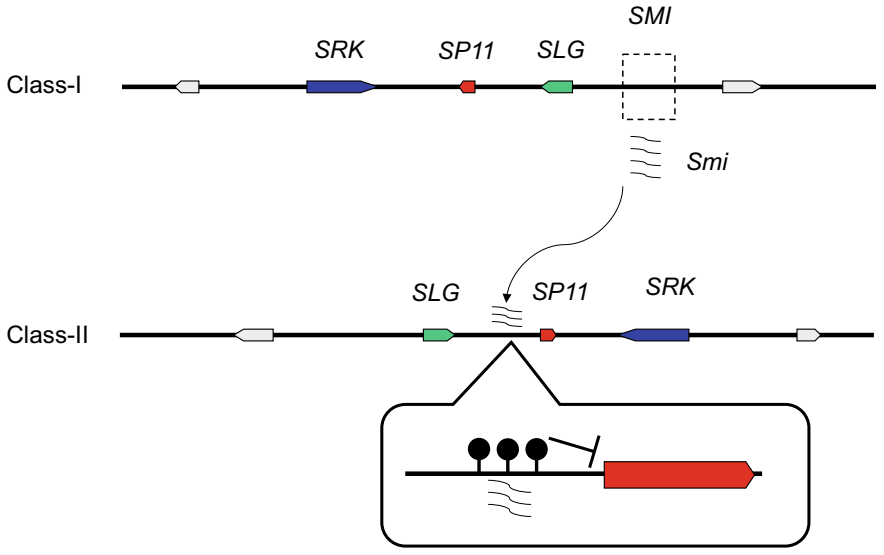

Fig. 3.31 Dominance relationship in pollen. *Smi* derived from class-I *S* locus can induce the de novo DNA methylation in the promoter region of class-II *SP11*. De novo DNA methylation silences the expression of class-II *SP11*

observed in the promoter region of recessive class-II *SP11* allele in the heterozygotes of class-II *S* haplotypes (Kakizaki et al. 2003; Yasuda et al. 2016). Like class-I/class-II heterozygotes, 24nt-small RNAs termed *SP11 methylation inducer 2* (*Smi2*) with sequence similarity to the promoter region of class-II *S* haplotypes are expressed, but they are expressed in all class-II *S* haplotypes. A linear dominance order in pollen is due to the sequence diversity within *Smi* among class-II *S* haplotypes; *Smi2* derived from dominant class-II *S* haplotype can bind to the promoter region of recessive class-II *S* haplotypes but *Smi2* derived from recessive class-II *S* haplotype cannot bind to the promoter region of dominant class-II *S* haplotypes because of nucleotide sequence difference (Fig. 3.32) (Yasuda et al. 2016).

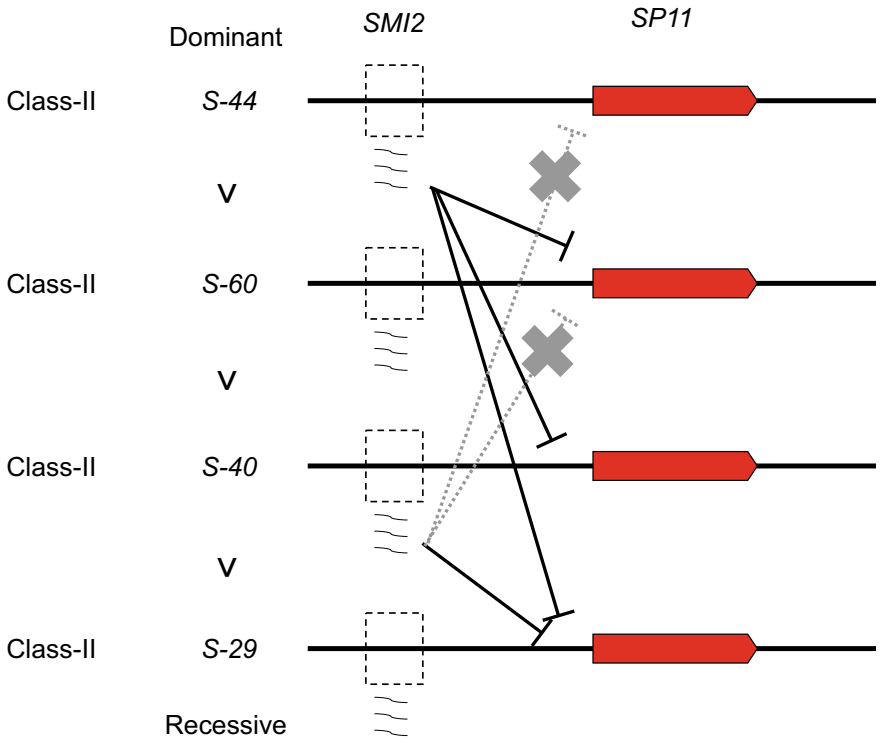

Fig. 3.32 Mode of action of the dominance relationships via *trans*-acting small RNA. The single *Smi2* regulates dominance hierarchy via a homology-dependent manner. In all dominant-recessive interactions, *Smi2* variants derived from dominant SMI2 region exhibited high similarity to the all-recessive *SP11* promoters and can induce the de novo DNA methylation leading to silencing of gene expression. By contrast, *Smi2* variants derived from recessive SMI2 region cannot induce the DNA methylation in the dominant *SP11* promoter because of low sequence similarity

3.4.6 S Haplotype Identification

Most cultivars of *Brassica* vegetables utilized for F_1 hybrid seed production system use self-incompatibility or cytoplasmic male sterility. As for the yield of F_1 hybrid seeds, F_1 hybrid breeding using the self-incompatibility system is much superior to that using male sterility. When self-incompatibility is used for harvesting F_1 hybrid seeds, identification of *S* haplotypes of breeding stocks is important for selecting parental combinations, thus avoiding the need for test crosses that are time-consuming. The method of *S* haplotype identification by DNA markers has been established by CAPS analysis using specific primer sets for amplification of *SLG*, or dot-blot analysis using high polymorphism of *SP11* alleles among *S* haplotypes (Nishio et al. 1996; Fujimoto and Nishio 2003; Oikawa et al. 2011). CAPS analysis using class-I and class-II *SLG*-specific primer pairs, PS5/15 and PS3/21, respectively, is well established to identify *S* haplotypes in the *Brassica* vegetables (Nishio et al. 1996), and *S* haplotypes in many cultivars in *B. rapa* and *B. oleracea* have been identified using this method (Sakamoto et al. 2000; Sakamoto and Nishio 2001; Park et al. 2001). This method is fully useful in the *B. rapa* vegetables; however, PCR products were not amplified in some *S* haplotype of *B. oleracea* vegetables (Kawamura et al. 2015, 2017). Similar problems are also observed in radish (Haseyama et al. 2018). It has been shown that some class-I *SLG* alleles could not be amplified using the primer set, PS5/15 (Nishio et al. 1996), and deletion of the *SLG* gene has been found in *B. oleracea* (Okazaki et al. 1999). Thus, it is necessary to use other strategies to distinguish the parental *S* haplotype. Another primer set, PK1/PK4, is also used for the identification of class-I *S* haplotype (Nishio et al. 1997), although no major improvement was seen for *B. oleracea* vegetables (Kawamura et al. 2017). Other primer sets, PSA/PSB (class-I *SLG*/*S* domain of *SRK*), HVR2-F/R (class-I *SLG*/*S* domain of *SRK*), and 60-F/40-R (class-II *SP11*), or combination of these primer sets may improve the identification of *S* haplotypes in *B. oleracea* vegetables. In the non-PCR-amplified *S* haplotypes by PS5/PS15 or PS3/PS21 primer set, there are sequence differences in the regions covering the primer; thus identification of the nucleotide sequence of *SLG* in these *S* haplotype is required for designing new primer sets suitable for *B. oleracea* vegetables as well as radish.

In an F_1 hybrid seed production system, high seed purity is essential. To confirm the purity of F_1 hybrid seeds, a field grow-out trial can be performed but it is time-consuming and laborious. Therefore, a DNA-marker-based purity test is useful, and identification of the *S* haplotype can be applied to a purity test (Fujimoto and Nishio 2007). Furthermore, SSR markers, which can distinguish the parental alleles of F_1 hybrid cultivars, could be applied for purity testing of F_1 hybrid seeds, and SSR markers have the advantage of being able to assess multiple markers, increasing the accuracy. Highly polymorphic SSR markers have been identified in *B. rapa* and *B. oleracea* (Kawamura et al. 2015, 2017).

3.4.7 Stability of Self-incompatibility

As above mentioned, self-incompatibility is used for harvesting F_1 hybrid seeds in *Brassica* vegetables, and high seed purity of F_1 hybrid seeds is essential for commercial use. However, instability of self-incompatibility influenced by environmental factors such as high temperature sometimes leads to production of low-quality seeds containing high percentage of selfed seeds. Given the future global climate change, stable and strong self-incompatibility is required for F_1 hybrid breeding. If there is an *S* haplotype showing stable or strong self-incompatibility, this *S* haplotype is useful as maternal line of F_1 hybrid cultivar. Though there are a few reports examining the strength of self-incompatibility, this strength is controlled by genetic background (Ruffio-Châble et al. 1997; Hatakeyama et al. 2010). Further study will be required for identification of the factors involved in the stability of self-incompatibility in the *Brassica* vegetables.

Strength of self-incompatibility is important for harvesting highly pure F_1 hybrid seeds. However, weakening or overcoming self-incompatibility is also important for development of inbred lines because inbred lines are commonly obtained by bud pollination, which is laborious. Therefore, various methods have been developed for overcoming self-incompatibility, and carbon dioxide treatment to self-pollinated flowers in a greenhouse or a plastic house is effective to reduce labors for seed production of the inbred lines (Nakanishi et al. 1969; Nakanishi and Hinata 1973). Yield of selfed seeds by bud pollination is generally low in the lines having strong self-incompatibility. Effect of genotype on response to CO_2 gas is also known (Nakanishi and Hinata 1973; Niikura and Matsuura 2000). By the genetic analysis, two major QTLs overcoming self-incompatibility during CO_2 gas treatments were identified and they did not link with the *S* locus (Lao et al. 2014).

The molecular basis of how self-pollen hydration, germination, or pollen tube elongation is inhibited is not fully understood. In addition, identification of factors involved in the stability of self-incompatibility will be required for the high purity of F_1 hybrid seeds, especially in the near future facing global climate changes. It is possible that factors involved in inhibition of self-pollen hydration, germination, or pollen tube elongation might be involved in the stability of self-incompatibility. Further progress of research in this filed is desired.

3.5 Genetic and Epigenetic Regulation of Flowering in the *Brassica* Vegetables

The changes from vegetative to reproductive growth mark a major developmental transition in flowering plants. Controlling the time of transition is important in the *Brassica* vegetables, because once the transition starts it cannot be reversed. Correct timing can maximize the reproduction success and seed production through ensuring the flowering time under optimal conditions. The late flowering or late bolting is

especially important for leafy *Brassica* vegetables, because premature bolting causes a decrease in productivity and market value. Therefore, much effort has been made in breeding programs to develop late bolting *Brassica* vegetable cultivars.

3.5.1 *Environmental Factors of the Regulation of Flowering Time*

Floral transition is highly responsive to environmental cues, and photoperiod and temperature play major roles (Srikanth and Schmid 2011). The regulation of flowering time, including its associated network, has been extensively studied in the model plant species *A. thaliana* (Putterill et al. 2004; Bäurle and Dean 2006; Fornara et al. 2010; Andrés and Coupland 2012; Song et al. 2013). More than 180 *A. thaliana* genes are recognized in flowering time control based on characterization of loss-of-function mutants or analysis of transgenic plants (Fornara et al. 2010). We know in *A. thaliana,* six major pathways control flowering time: the photoperiod/circadian clock pathway, vernalization pathway, ambient temperature pathway, age pathway, autonomous pathway, and gibberellin pathway (Fig. 3.33) (Kim et al. 2009; Fornara

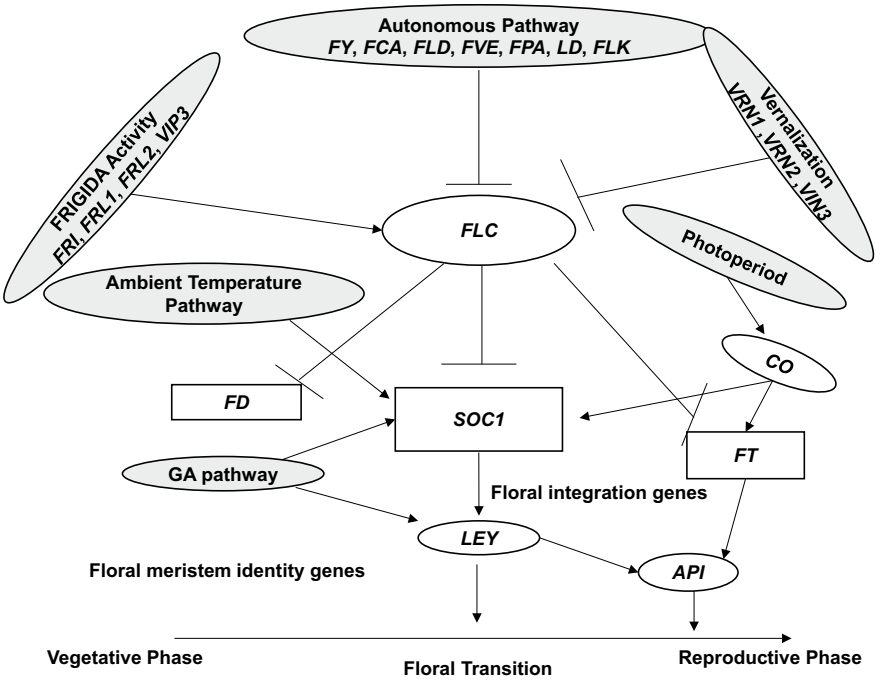

Fig. 3.33 A simplified schematic showing *FLOWERING LOCUS C* (*FLC*), involving a complex network pathway for flowering in *A. thaliana*

et al. 2010). Among them, the photoperiod response to changes in day length and the vernalization response to low temperatures are two major pathways that regulate flowering time in *A. thaliana* (Song et al. 2013*)*. Other pathways are able to modulate the flowering response like the ambient temperature pathway, the age pathway, the sugar signaling pathway, and the stress pathway (Srikanth and Schmid 2011; Blümel et al. 2015).

Various numbers of genes and micro-RNAs (miRNAs) are involved in the regulation of flowering time, which help us to understand the involvement of these factors at the molecular level. Mainly, the photoreceptor proteins (phytochrome and/or cryptochrome) are controlling the photoperiodism, which is responsible for sensing red/far-red and blue light, respectively (Más et al. 2000). Photoperiod requirements are defined as either long day (LD) or short day (SD) with respect to the length of time of daylight. This photoperiod signal plays vital role in the floral development of several plant species, which is related to the annual cyclical seasonal changes, LD, coinciding with the spring and summer seasons, and SD, associated with the autumn and winter seasons, respectively (Corbesier and Coupland 2005).

Vernalization is defined as "the acquisition or acceleration of the ability to flower by a chilling treatment." In *A. thaliana*, the prolonged exposure to cold will decrease the *FLOWERING LOCUS C* (*FLC*) expression, which acts as a floral repressor by inhibiting the activation of a set of genes required for transition of the apical meristem to a reproductive state (Kardailsky et al. 1999; Kobayashi et al. 1999; Michaels and Amasino 1999; Sheldon et al. 1999; Lee et al. 2000; Samach et al. 2000; Hepworth et al. 2002). Vernalization is an example of temperature-accelerated flowering (Song et al. 2012). When other specific conditions are met, including the presence of certain photoperiods and ambient temperatures, and vernalization, flowering only takes place many weeks or even months later (Kim et al. 2009).

B. rapa and *B. oleracea* show different responses to vernalization; *B. rapa* responds to seed vernalization, whereas *B. oleracea* requires plant vernalization (Lin et al. 2005). In seed-vernalization-responsive type, plants can sense low temperatures during seed germination. On the other hand, in plant-vernalization-responsive type, plants need to reach a certain developmental stage before they become sensitive to low temperatures (Friend 1985). In the plant-vernalization-responsive type, plants grow vegetative in the first year and flower in the following year after winter. *B. napus* is an important oilseed crop; natural variation in flowering time in response to vernalization was characterized into three groups (spring, winter, and semi-winter type) (Raman et al. 2016). Spring-type varieties are annual type generally seeded in spring and complete their life cycle in a single growing season without vernalization; winter (biennial) types have an obligate requirement usually seeded in the fall and complete development in the following spring under prolonged period of cold temperature. Semi-winter types are sown before winter, which gives flower after winter.

3.5.2 *Photoperiod and Circadian Clock Mechanism in the Brassicaceae*

The circadian clock mechanism controls the flowering time in concert with the photoperiodic flowering pathway (Jung and Müller 2009; Imaizumi 2010; Song et al. 2013, 2015). By the circadian clock mechanisms in LD condition, *A. thaliana* perceives LD light in the leaves, which involve the *CONSTANS* (*CO*), *GIGANTEA* (*GI*), and *FLAVIN KELCH F BOX 1* (*FKF1*) genes. The interaction of upstream genes of *CO* such as *GI* and *FKF1* releases repression of *CO* transcription by inducing degradation of the transcriptional repressor *CYCLING DOF FACTOR1* (*CDF1*) (Srikanth and Schmid 2011). The transcription and protein function of *CO* tightly controlled by the light and circadian clock genes controls floral activator *FLOWERING LOCUS T* (*FT*) expression to induce flowering via the photoperiod pathway (Corbesier et al. 2007). *FT* expresses within the distal part of the leaf and moves through the phloem to the meristem acting as a long-distance systemic signal between leaves and the shoot meristem (Kardailsky et al. 1999; Weigel et al. 2000; Turck et al. 2008). FT interacts with the bZIP transcription factor (TF) FLOWERING LOCUS D (FD) to form a FT/FD heterodimer complex in the shoot apical meristem (SAM) (Abe et al. 2005; Wigge et al. 2005), which activates expression of the floral meristem identity genes, *APETALA 1* (*AP1*) and *FRUITFUL* (*FUL*), thus initiating the development of flower buds (Abe et al. 2005; Wigge et al. 2005; Corbesier et al. 2007; Turck et al. 2008; Turnbull 2011).

As a main component of the clock, CIRCADIAN CLOCK ASSOCIATED 1 (CCA1), a MYB-related TF, which plays an important role in the phytochrome-dependent induction of photosynthetic genes (Wang et al. 1997; Green and Tobin 2002), controls the circadian clock (Green and Tobin 2002; McClung 2014), stress response (Dong et al. 2011; Lai et al. 2012; Seo et al. 2012), and maintenance of photoperiodic flowering (Niwa et al. 2007; Fujiwara et al. 2008). Another core component of the circadian clock is LATE ELONGATED HYPOCOTYL (LHY) in *A. thaliana* (Wang et al. 1997; Wang and Tobin 1998), which is involved in the regulation of photoperiodic flowering (Fujiwara et al. 2008; Imaizumi 2010; Li et al. 2011; Lu et al. 2012).

In *B. rapa,* preferential retention is more important for *CCA1* gene like *CCA1/LHY/RVE* and *PRR* gene families, but not *ZTL/FKF1/LKP2* families because they are not retained in *B. rapa* genome (Lou et al. 2012). *B. rapa* has two copies of *Bra.FT* and three copies of *Bra.CO* (Zhang et al. 2015). In contrast, *B. oleracea* seems to carry four copies of *Bol.FT* and three copies of *Bol.CO* (Razi et al. 2008). *Bra.FT.A07*, often referred to as *BrFT2*, has a transposon insertion in the mapping parent R-o-18 and underlie a strong QTL for flowering time (Zhang et al. 2015). In a DH population derived from a Chinese cabbage and a rapid cycling line, a *CO*-like copy on A02 co-localized with a flowering QTL (Li et al. 2013b).

3.5.3 Vernalization Requirement and Responses in the Brassica Vegetables

In *A. thaliana,* mainly two key genes, *FRIGIDA* (*FRI*) and *FLC*, have been identified; *FLC* blocks flowering by binding to genes that promote flowering and repressing their transcription. Mainly FLC targeted three flowering time genes, *FT, SOC1,* and *FD*, with FLC binding to the promoters of *SOC1* and *FD* and to the first intron of *FT* (Helliwell et al. 2006; Searle et al. 2006). Later, at the whole-genome level, more putative *FLC* targeted genes were identified by ChIP-seq. Five-hundred *FLC* binding sites were found, mostly located in the promoter region of genes containing one CArG box (the known target of MADS-box proteins) (Deng et al. 2011). In the photoperiod pathway, two genes (*FT* and *SOC1*) act downstream of the flowering activator CO that is being negatively regulated by FLC (Kim et al. 2009; Andrés and Coupland 2012).

Plant homeodomain (PHD) finger protein (*VERNALIZATION INSENSITIVE 3,* VIN3) induces during the exposure to cold, which acts to establish the initial repression of *FLC* (Sung and Amaniso 2004). Moreover, VIN3, VRN5, and VIN3/VRN5-like 1 (VEL1) interact with VRN2 protein and form PHD-PRC2 complex (Sung and Amaniso 2004; Wood et al. 2006; De Lucia et al. 2008). Vernalization reduces the *FLC* repression, which is associated with the enrichment of H3K27me3 mediated by the PHD-PRC2 mechanism (De Lucia et al. 2008). During exposure to cold, H3K27me3 is enriched in chromatin at the transcription start sites of *FLC*, and later H3K27me3 modification extends across the *FLC* gene due to warm temperature (Finnegan and Dennis 2007). A stable maintenance of repression requires PRC2, although the initial transcriptional repression of *FLC* is PRC2-independent (Gendall et al. 2001). After cold exposure, the maintenance of *FLC* silencing under warm conditions is therefore mediated by PHD-PRC2 spreading H3K27me3 over the *FLC* locus. Additionally, LIKE HETEROCHROMATIN PROTEIN 1 (LHP1), associated with H3K27me3, and VRN1 are also required for the maintenance of stable *FLC* repression (Levy et al. 2002; Mylne et al. 2006; Sung et al. 2006).

In *B. rapa*, several QTLs were identified for flowering time (VFR1, 2, and 3 in non-vernalized condition and FR1, 2, and 3 in vernalized condition) from a cross between an annual and a biennial oilseed cultivar (Teutonico and Osborn 1994; Osborn et al. 1997), which covers the region of *BrFLC1* and *BrFLC2* (Kole et al. 2001; Schranz et al. 2002). Eight QTLs for flowering with one major QTL, which co-localized with *BrFLC2,* were detected using a multi-population derived from several parental lines (rapid cycling, Chinese cabbage, yellow sarson, pak choi, and a Japanese vegetable turnip variety) (Lou et al. 2007). QTL analyses also showed the co-localization of a major QTL with *BrFLC2* using other parental combinations between pak choi and yellow sarson (Zhao et al. 2010; Xiao et al. 2013). Over many years' QTL analysis has shown a major QTL of flowering time co-localized with *BrFLC2*. QTL analysis was performed in two different conditions, greenhouse and open field using an F_2 population derived from a cross between an extremely late bolting line (Nou 6 gou and PL6) and early bolting line (A9709) of Chinese cabbage. Five QTLs were detected,

but within two condition QTLs did not map in the same position. Among the five QTLs, three QTLs were co-localized with *BrFTa* (greenhouse), *BrFLC1* (open field), and *BrFLC5* (open field) (Kakizaki et al. 2011). In Chinese cabbage, an F_2 population was derived from the cross of an early bolting commercial F_1 varieties, "Early", and an extremely late bolting line, "Tsukena No. 2", where QTLs for bolting time after vernalization co-localized with the late bolting alleles of *BrFLC2* and *BrFLC3*. In the extremely late bolting of "Tsukena No. 2", large insertions were found in the first intron of *BrFLC2* and *BrFLC3*, suggesting that weak repression of *BrFLC2* and *BrFLC3* transcripts by vernalization results in these insertions (Kitamoto et al. 2014). In addition, this group successfully developed new F_1 hybrids of Chinese cabbage by introducing these two *FLC* alleles from Tsukena No. 2 (Kitamoto et al. 2017).

In *B. oleracea*, QTL analysis identified a major QTL covering *BoFLC2*, while *BoFLC1*, *BoFLC3*, and *BoFLC5* were not linked, using a population derived from a DH line of broccoli, Green Comet, and a DH line of cabbage, Reiho (Okazaki et al. 2007). Due to deletion of a single base in exon 4 leading to a frameshift mutation, suggesting that *BoFLC2* contributes to the control of flowering time in Green Comet (non-vernalization type) (Okazaki et al. 2007). Another group conducted QTL analysis using the population derived from a rapid cycling line of *B. oleracea* var. *alboglabra* (A12DHd) and the broccoli variety, Green Duke. *BoFLC2* is not responsible for the flowering time difference between the two lines because these two lines bear nonfunctional copies of *BoFLC2*; there is a single base deletion in exon 4 and deletion in the A12DH in Green Duke (Razi et al. 2008). The association between flowering time (under both glasshouse and field conditions) and a QTL at *BoFLC2* has been shown using the population of two purple sprouting broccoli lines (E5 and E9); E9 requires longer cold periods than E5 to head (Irwin et al. 2016). Through allelic variation and sequence polymorphisms, *BoFLC2* was shown to be a major determinant of heading date variation and vernalization response and alters the sensitivity and silencing dynamics of its expression (Irwin et al. 2016). In *B. rapa*, hybridized introgression of *BoFLC2* from a plant-vernalized *B. oleracea* cultivar did not alter the vernalization phenotype in the derived BC_3F_2 offspring; however, the duration of cold required for successful vernalization leading to flowering was increased, suggesting that the duration of cold experienced is altered by allelic variation, while the difference in the developmental stage at which vernalization will occur between the two species is possibly controlled by another mechanism (Shea et al. 2017, 2018c).

In *B. rapa*, after vernalization, the expression of *BrFLC* genes was reduced and is stably maintained after returning to ambient temperatures (Fig. 3.34). During normal growth, three of the *BrFLC* paralogs (*BrFLC2, BrFLC3*, and *BrFLC5*) showed H3K4me3 modification, while only *BrFLC1* showed accumulation of both H3K4me3 and H3K36me3. After 4 weeks of vernalization, the accumulation of H3K27me3 was observed in *BrFLC1, BrFLC2*, and *BrFLC3,* and maintained after returning to a warm temperature (Kawanabe et al. 2016a); the repression of *BrFLC* expression by prolonged cold treatment was associated with the histone modification. Previous studies of *A. thaliana*, long noncoding RNAs (lncRNAs) such as COLD-INDUCED LONG ANTISENSE INTRAGENIC RNA (COOLAIR), COLD-ASSISTED INTRONIC

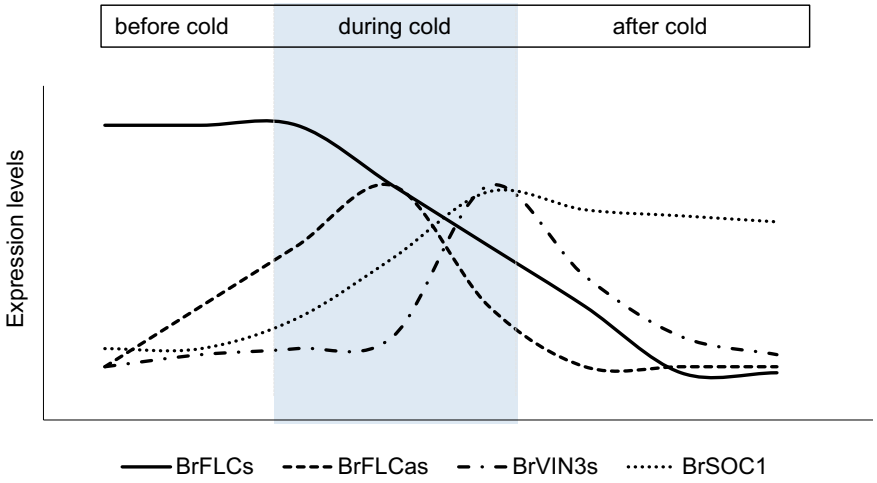

Fig. 3.34 Schematic illustration of gene expression levels of genes involved in response to vernalization in *B. rapa*

NONCODING RNA (COLDAIR), and COLD OF WINTER-INDUCED NONCODING RNA FROM THE PROMOTER (COLDWRAP) are involved in vernalization (Swiezewski et al. 2009; Heo and Sung 2011; Kim and Sung 2017). Cold-induced lncRNA COLDAIR is expressed from the mid-region of the first intron (Tsai et al. 2010; Heo and Sung 2011). In *A. thaliana*, the first intron, the promoter region, and exon 1 are important for the regulation of *FLC* expression by prolonged cold treatments (Sheldon et al. 2002). Although in *B. rapa* long insertions in the first intron cause a weak repression of *BrFLC2* and *BrFLC3* transcripts by vernalization, sequence similarity to the vernalization response element (VRE) in the first intron or to the COLDAIR of *A. thaliana* was not detected in the first intron of any of the *B. rapa* paralogs (Kitamoto et al. 2014). COLDAIR-like transcripts were not detected, but COOLAIR-like transcripts were detected only from *BrFLC2*, and these transcripts were induced by cold treatment in *B. rapa* (Li et al. 2016e).

High bolting resistance is an important trait for cultivation mainly in leafy vegetables such as Chinese cabbage or cabbage, which requires a deep understanding of the molecular mechanism to control the vernalization requirement. COOLAIR-like transcripts were detected only from *BrFLC2,* which regulated the suppression of *BrFLC2* and maybe other *BrFLC*s (Li et al. 2016e), but in *B. rapa*, there is no report found about the transcripts of COLDAIR or COLDWRAP, and regions sharing sequence similarity to the COLDAIR (Kitamoto et al. 2014). Therefore, there is a possibility that lncRNAs may be involved in the regulation of repression of *FLC,* which will need to be assessed by RNA-seq. Thus, in the genus *Brassica*, it is important to identify the sequences required for vernalization, termed VREs, and to examine any sequence polymorphisms that may help to identify important regions and develop their relationship to sensitivity of vernalization; this will be helpful for

marker-assisted selection (MAS) and serve as important tools for breeding in the genus *Brassica*.

3.5.4 Perspective

Future climatic shifts will affect the flowering time over the coming decades. Therefore, future research into flowering time and the various interconnected regulatory pathways continues to remain an invaluable source of information for the application of MAS and the continued development of hardy crop breeds. The current understanding of vernalization response and heading time, and its connection to the *FLC* haplotype is therefore an important consideration in the breeding of cole crops as climates begin to respond to an increased mean global temperature. In stable climatic regions, the breeding of such crops is well understood. The additional challenges of a changing climate, however, increase the demands placed upon both breeders and agricultural researchers.

3.6 Disease Resistance Genes in the *Brassica* Vegetables

Climate change, pathogen variations, and inappropriate farming methods are posing threat to current production of *Brassica* vegetables. Various pathogens, including virus, bacteria, fungi, and oomycete, can infect *Brassica* vegetables leading to production loss. Among the diseases, turnip mosaic virus, black rot, Fusarium wilt, and clubroot have been focused due to their impact on farming.

Traditional methods of disease prevention include physical, chemical, and biological control. Physical methods are often complicated and are not effective compared to other methods. Besides, chemical and biological controls may have effects under certain conditions but are costly and/or environmentally damaging. In contrast, natural resistance from *Brassica* hosts is the most desirable method of disease prevention.

There are two types of plant immunity: (1) pathogen/microbe-associated molecular patterns (PAMPs/MAMPs) triggered immunity (PTI) activated through recognition of PAMPs/MAMPs by cell surface pattern recognition receptors (PRRs), and (2) effector-triggered immunity (ETI) activated through the recognition of pathogen-specific effector molecules by host resistance (*R*) genes, which reflects the "gene-for-gene" theory (Flor 1971; Chisholm et al. 2006). Most *R* genes encode nucleotide-binding leucine-rich repeat (NB-LRR) proteins, including coiled-coil NB-LRRs (CC-NB-LRRs) and toll interleukin-1 receptor-NB-LRRs (TIR-NB-LRRs). Some *R* genes also encode transmembrane receptor-like proteins (RLPs), transmembrane receptor-like kinases (RLKs), cytoplasmic kinases (CKs), and proteins with atypical molecular motifs (Jones and Dangl 2006; Liu et al. 2007; Neik et al. 2017). In recent years, a variety of *R* genes has been identified and successfully applied to improve the resistance against various diseases in the *Brassica* vegetables.

3.6.1 Turnip Mosaic Virus (TuMV)

The *Brassica* infecting viruses mainly include turnip mosaic virus (TuMV), cucumber mosaic virus (CMV), tobacco mosaic virus (TMV), and cauliflower mosaic virus (CaMV) with TuMV being the most prevalent and causing the greatest loss for *Brassica* crops. TuMV is a member of the virus genus *Potyvirus*. The disease was first described in 1921 in USA in *B. rapa* and then in *B. oleracea* in the UK (Smith 1935). Now, TuMV is threatening *Brassica* vegetables around the world, especially in Europe, Asia, and North America, resulting in a yield loss up to 30% (Tomlinson 1987; Walsh and Jenner 2002). The diseased plants show symptoms including slight leaf stunting, mottle, and chlorosis, or spot in the primary infection stage, and severe stunting, chlorosis, necrosis, and even withering of the whole plant in the late infection stage; during subsequent cold storage of the leaf head in *Brassica*, internal necrosis further develops and makes the leaf heads unmarketable. TuMV is difficult to control, because of its wide host range and high pathotype diversity, and has caused serious losses in almost all *Brassica* vegetables, as well as many non-*Brassica* vegetables such as radish, pea, and lettuce (Provvidenti 1980). Based on differential hosts or molecular variations, TuMV strains or pathotypes have been defined to strains C1-4 (Provvidenti 1980), C1-5 (Green and Deng 1985), pathotypes 1-12 (Walsh 1989; Jenner and Walsh 1996), four phylogenetic groups including basal-B, world-B, basal-BR, and Asian-BR (Ohshima et al. 2002, 2007), or four host types including B(B), BR, and B(R) (Tomimura et al. 2004; Tomitaka and Ohshima 2006; Nguyen et al. 2013). Another reason for the difficulty of control is the non-persistent mode of transmission by at least 89 aphid species (Hamlyn 1953; Walsh and Jenner 2002). Traditional methods like chemical control are not effective and environmentally damaging, while natural resistance from *Brassica* hosts is the most desirable way of preventing TuMV. In recent years, a variety of *R* genes/loci has been identified and successfully applied to improve the resistance against TuMV in the *Brassica* vegetables.

TuMV resistance has been identified in most *Brassica* species (Doucet et al. 1990; Fjellstrom and Williams 1997; Walsh et al. 2002; Nyalugwe et al. 2014, 2015a, b). Extensive studies have revealed various inheritance types of TuMV resistance, which indicated a gene-for-gene system. Generally, the resistance genes are mostly found on the A genome, being dominant or recessive and displaying a qualitative genetic control by one or two genes, while the resistance on the C genome is mostly inherited in polygenic manner. In *B. rapa*, there are both dominant and recessive inheritance types. Suh et al. (1995) showed that the resistance to single strain was conferred by one or two dominant genes, while resistance to mix strains was controlled by more than two major effect genes. In recent years, new single dominant genes were identified by genetic analysis using different DH, F_2, or BC segregation populations (Chung et al. 2014; Jin et al. 2014; Li et al. 2015c). Recessive inheritance type of the resistance was also discovered. Yoon et al. (1993) reported that Chinese cabbage 0–2 line's resistance to strain C4 and C5 was regulated by two recessive genes. Several other single recessive genes have also been identified, including *recessive Turnip*

mosaic virus resistance 01(*retr01*) (Rusholme et al. 2007), *resistance and necrosis to tumv 1*(*rnt1*) (Fujiwara et al. 2011), *retr02* (Qian et al. 2013), *trs* (Kim et al. 2013), and *retr03* (Shopan et al. 2017). In *B. oleracea*, most studies have revealed dominant genetic structure. In Brussel sprouts, at least four genes control the resistance to TuMV (Pink et al. 1986). Pink and Walkey (1990) further analyzed the inheritance of disease resistance using new cabbage materials, and the estimated heritability of resistance ranged from 41 to 48%.

More than ten *R* genes to TuMV have been identified in the genus *Brassica* (Table 3.2). *Turnip mosaic virus RESISTANCE IN BRASSICA 01* (*TuRB01*), a single dominant resistance gene to pathotype 1, was first mapped by Walsh et al. (1999) to a 7.2 cM interval on chromosome N6 of *B. napus*. *TuRB01b* was identified on a 2.9 Mb region of chromosome A06 from *B. rapa*, and comparative analysis indicated that *TuRB01* and *TuRB01b* represent similar or identical alleles at the same A genome resistance locus (Lydiate et al. 2014). *TuRB02*, identified on the *B. napus* C genome linkage group (LG) N14, controls the degree of susceptibility to isolate CHN1 (Walsh et al. 1999). *TuRB03*, a single dominant gene conferring resistance to pathotype 4, was mapped to a 7.9 cM interval on chromosome N6 in *B. napus* (Hughes et al. 2003). *retr01* represents the first mapped recessive gene in *Brassica* species and was mapped on chromosome R4 (Rusholme et al. 2007); another recessive gene *rnt1* from *B. rapa* was mapped on chromosome R6 (Fujiwara et al. 2011). Besides, Li et al. (2015c) mapped a novel *B. rapa* resistance gene *TuRBCS01* to a 1.98 Mb region on chromosome A04 using SSR and InDel markers. Using bulked segregation analysis (BSA), Shopan et al. (2017) identified another single recessive gene *retr03*. The previous mapping work opened the gate for isolation and analysis of the candidate *R* genes. The dominant gene *ConTR01* and the recessive genes *retr01*, *retr02*, and *retr03* were all supposed to be eIF4E or eIF(iso)4E encoding genes (Rusholme et al. 2007; Qian et al. 2013; Shopan et al. 2017). *BcTuR3*, isolated from non-heading Chinese cabbage, was a TIR-NB-LRR type *R* gene related to TuMV resistance (Ma et al. 2010). *TuMV-R* from *B. rapa* was mapped to a 0.34 Mb region on chromosome A06, with containing six candidate genes (Chung et al. 2014). *TuRB07*, a single dominant gene from *B. rapa*, was mapped to chromosome A06, and the candidate gene is Bra018863 (encoding CC-NB-LRR) (Jin et al. 2014).

The protein–protein interaction in the *Brassica*–TuMV system has also received much attention. A variety of protein interactions has been characterized till now, using techniques such as yeast two-hybrid (Y2H), bimolecular fluorescence complement (BiFC) and co-immunoprecipitation (CoIP). Researchers have identified the cytoplasmic inclusion (CI) protein as the interactor and viral avirulence determinant for TuRB01, TuRB01b, and TuRB04, while P3 is the viral avirulence determinant for TuRB03 and TuRB05 (Jenner et al. 2000, 2002, 2003; Walsh et al. 2002). Another example is the plant eukaryotic initiation factor 4E (eIF4E) family, a well-known host factor that plays a critical role in the infection of several potyviruses. The interaction between viral protein genome-linked (VPg) of potyviruses and eIF4E or eIF(iso)4E of the host determines the virulence (Wittmann et al. 1997; Robaglia and Caranta 2006; Beauchemin et al. 2007). This eIF4E-mediated resistance often confers strong and broad-spectrum resistance (Yeam et al. 2007; Rodríguez-Hernández et al. 2012).

Table 3.2 Mapped or cloned TuMV resistance genes in the genus *Brassica*

R genes	Species	Inheritance and mapping results	Pathotype/Strains	References
TuRB01	*B. napus*	Single dominant; mapped to a 7.2 cM interval on N6	1	Walsh et al. (1999)
TuRB02	*B. napus*	Dominant; mapped to N14	CHN1, JPN1	Walsh et al. (1999)
TuRB03	*B. napus*	Single dominant; mapped to a 7.9 cM interval on N6	CDN1	Hughes et al. (2003)
TuRB04	*B. napus*	Single dominant; unmapped	1, 3	Jenner et al. (2002, 2003)
TuRB05	*B. napus*	Single dominant; unmapped	1, 3	Jenner et al. (2002, 2003)
retr01	*B. rapa*	Recessive; mapped to R4, and the candidate is an eIF4E encoding gene	1, 3, 4, 7, 8, 9, 12	Rusholme et al. (2007)
ConTR01	*B. rapa*	Dominant; mapped to R8, and the candidate is an eIF(iso)4E encoding gene	1, 3, 4, 7, 8, 9, 12	Rusholme et al. (2007)
BcTuR3	*B. rapa*	Possibly related gene, TIR-NB-LRR type	–	Ma et al. (2010)
rnt1	*B. rapa*	Recessive; mapped on R6,	UK1	Fujiwara et al. (2011)
retr02	*B. rapa*	Recessive; mapped to A4, candidate is Bra035393, encoding eIF(iso)4E	C4	Qian et al. (2013)
trs	*B. rapa*	Recessive; mapped to A4.	CHN2, 3, 4, 5	Kim et al. (2013)
TuMV-R	*B. rapa*	Mapped to a 0.34 Mb region on A6, with six candidates	–	Chung et al. (2014)
TuRB01b	*B. rapa*	Single dominant; mapped to a 2.9 Mb region on A06	1	Lydiate et al. (2014)
TuRB07	*B. rapa*	Single dominant; mapped to A6, candidate gene is *Bra018863* (CC-NB-LRR)	C4	Jin et al. (2014)

(continued)

Table 3.2 (continued)

R genes	Species	Inheritance and mapping results	Pathotype/Strains	References
TuRBCS01	*B. rapa*	Mapped to a 1.98-Mb region on chromosome A04	C4	Li et al. (2015c)
TuRBJU01	*B. juncea*	Dominant; unmapped	8	Nyalugwe et al. (2016)
retr03	*B. juncea*	Recessive; encoding eIF2Bβ	ZJ strains	Shopan et al. (2017)

In the genus *Brassica*, the eIF(iso)4E-encoding gene has been shown to be strongly linked to the recessive resistance genes *retr01*, *retr02,* and *trs* (Rusholme et al. 2007; Qian et al. 2013; Kim et al. 2013), and transgenic plants overexpressing *eIF(iso)4E* variants show broad-spectrum TuMV resistance (Kim et al. 2014). Except for the direct application of the identified resistance genes, the genes from TuMV have also been used in resistance breeding by host-induced gene silencing (HIGS), especially the *coat protein* (*CP*) gene derived from the virus. The viral CP can accumulate in the host cells and inhibit the virus replication, and thus confers resistance. Successful resistance enhancement by *CP* gene strategy has been reported on *Brassica* crops including oilseed rape and Chinese cabbage (Jan et al. 2000; Lehmann et al. 2003).

Although great progress has been made in terms of genetic mapping of the *Brassica*–TuMV resistance genes, these candidate genes still require further functional analysis, and the results from TuMV-*Arabidopsis* systems could provide evidence (Martín et al. 1999; Liu et al. 2015). Till now, there are few studies concerning other *Brassica*-affecting viruses including CMV, TMV, CaMV, etc. However, the progress made in TuMV in *Brassica* crops and CMV and TMV in other crops may open the gate for these future studies.

3.6.2 Black Rot (BR)

Bacterial diseases for the genus *Brassica* include black rot (BR) caused by *Xanthomonas campestris* pv. *campestris* (*Xcc*), soft rot caused by *Erwinia carotovora* subsp. *carotovora*, and leaf black spot caused by *Alternaria oleracea*, among which BR brought about the greatest loss and has been studied most extensively. *Xcc* belongs to the genus *Xanthomonas*, including many economically important pathogenic bacteria associated with plants. The disease has been identified in all *Brassica* growing continents, especially in Asia, Europe, and North America (Jensen et al. 2010; Singh et al. 2016). The pathogen usually invades through hydathodes or wounds, and spreads into the leaf and the whole plant through the vascular system (Vicente and Holub 2013). The disease starts as chlorotic lesions in the leaf margins, and the pathogen reproduces and its secretion blocks the vessels and water transport, resulting

in V-shaped lesions and dark veins, and finally, the whole plant wilts leading to death (Schaad 1982; Alvarez et al. 1994). *Xcc* has a wide host range and can cause serious damage on *B. oleracea*, as well as other *Brassicaceae* crops, radish, ornamental crucifers, related weed species, and even *A. thaliana* (Bradbury 1986). *Xcc* has a high diversity and 11 races have been identified with race 1 and 4 being prevalent (Vicente et al. 2001; Fargier and Manceau 2007; Cruz et al. 2017). BR is a seed-borne disease, and the pathogen is mainly transmitted through contaminated seeds and transplants (Vicente and Holub 2013). BR can easily become epidemic under some favorable conditions like high humidity and warm temperature (Staub and Williams 1972; Vicente and Holub 2013). Countermeasures including seed treatment, soil disinfection, crop rotation, and biocontrol agents have some effects (Massomo et al. 2004). The development and use of resistant cultivars have long been considered as important methods of disease control (Taylor et al. 2002; Lee et al. 2015). In recent years, the inheritance of BR resistance has been studied in several *Brassica* species and some QTLs have been mapped using molecular markers (Vicente et al. 2001; Taylor et al. 2002).

Many studies focused on *Brassica* crops. However, only few resistance sources have been identified, including two extensively utilized cabbage accessions "Early Fuji" from Japan and PI436606 (cultivar Heh Yeh da Ping Tou) from China (Hunter et al. 1987; Camargo et al. 1995). Most studies for BR resistance were performed on *B. oleracea* crops and revealed complex genetic structures. Using F_2 and BC populations, Jamwal and Sharma (1986) showed that the BR resistance in the cauliflower cultivar SN445 was controlled by a dominant gene. Badger Inbred 16, a line derived from "Early Fuji", contains QTLs for BR resistance (Camargo et al. 1995; Vicente et al. 2002). Ignatov et al. (1998) found in progenies of cabbage line PI436606, Portuguese kale ISA454, and Chinese kale SR1, that high resistance to race 1 was controlled by a dominant gene named *R1*, when a recessive gene *r5* was responsible for the resistance to race 5. Vicente et al. (2002) found resistance to race 3 in the cabbage DH line BOH 85c and in PI436606 was controlled by a single dominant locus (*Xca3*). Recently, Saha et al. (2014, 2016) found that resistance to race 1 in cauliflower accession BR-207 was governed by a single dominant gene. In view that there are few highly resistant resources in C genome of *B. oleracea,* especially to the prevalent race 1 and 4, researchers tend to screen for useful sources from the A and B genomes. The gene conferring resistance to race 1 is present in the B genome of *B. carinata*, *B. juncea,* and *B. nigra*, while the gene conferring resistance to race 4 is present in the A genome of *B. rapa*, *B. napus,* and *B. juncea* (Taylor et al. 2002). Vicente et al. (2002) found that strong resistance to races 1 and 4 was controlled by a single dominant locus *Xca1* in the *B. carinata* line PI199947, while resistance to race 4 in three *B. napus* lines was controlled by a single dominant locus (*Xca4*). Major dominant inheritance type in *B. carinata* was also proved in other studies (Guo et al. 1991; Sharma et al. 2016). Griffiths et al. (2009) identified five *B. rapa* accessions with variable resistance to race 1 and uniformly resistance to race 4, all of them having the oilseed plant growth type.

Most BR resistance research focused on QTL analysis or preliminary mapping. The first QTL analysis of BR resistance in Badger Inbred 16 using RFLP markers

has revealed two major QTLs on LGs 1 and 9 (Camargo et al. 1995). Vicente et al. (2002) used *B. napus* DH populations and positioned the locus *Xca4* on LG N5 of the A genome. Soengas et al. (2007) reported the genetics of broad-spectrum resistance in the Chinese cabbage accession B162, and resistance to both race 1 and 4 correlated and a cluster of highly significant QTL that explained 24–64% of the phenotypic variance was located on chromosome A06. To analyze resistance in cabbage line "Reiho", Doullah et al. (2011) adopted sequence-related amplified polymorphism (SRAP) and CAPS markers and performed QTL analysis with $F_{2:3}$ families, and revealed QTLs on LG2 accounting for up to 10% of the phenotypic variation and another one on LG9 explaining 16% phenotypic variation. The high-throughput markers allow more accurate mapping. Kifuji et al. (2013) used expressed sequence tag (EST)-based SNP markers to map the resistance gene in "Early Fuji" to race 1, and three QTLs, i.e., *QTL-1* (the major one), *QTL-2*, and *QTL-3*, were detected. Tonu et al. (2013) analyzed BR resistance QTLs, and the major QTL *XccBo(Reiho)2* was detected on chromosome C8. Saha et al. (2014) mapped *Xcc* race 1 resistance gene *Xca1bo* in cauliflower line BR-161, with the 1.6 cM interval being flanked by one RAPD marker and one inter-simple sequence repeat (ISSR) marker. Kalia et al. (2017) further converted these markers to sequence characterized amplified region (SCAR) markers and proved that these markers were useful in MAS in cauliflower breeding. Sharma et al. (2016) firstly developed *B. carinata* F_2 mapping population and intron length polymorphic markers, to map the BR race 1 resistance locus *Xca1bc* in a 6.6 cM interval. Lee et al. (2015) firstly developed genome-wide SNP markers based on resequencing data and identified one major QTL on chromosome C03 in cabbage. In total, more than 20 QTLs with major or minor effects have been mapped on eight different *Brassica* chromosomes, suggesting that resistance to BR disease is complex and quantitatively controlled by multiple genes (Table 3.3).

Current molecular and omics methods provide new opportunities for quick disease-related gene mining. Jiang et al. (2011) investigated the molecular resistance mechanisms to find the genes related to BR resistance in cauliflower, using suppression subtractive hybridization (SSH) technique. The results imply that some upregulated genes might be involved in cauliflower resistance responses, such as plant defensin PDF1.2, lipid transfer protein, and thioredoxin h. Tortosa et al. (2018) firstly investigated the dynamic changes in the metabolic profile of *B. oleracea* plants during an *Xcc* infection from leaves and found specific metabolic pathways such as alkaloids, coumarins, or sphingolipids are postulated as promising key role candidates in the infection response. Using RNA-seq, Afrin et al. (2018) revealed that six NB-encoding genes were highly expressed in resistant cabbage lines compared to susceptible cabbage lines, which were possibly related to BR resistance.

Although some *R* genes display single inheritance pattern, none has been cloned and functionally analyzed. Thus, more efforts will be needed on these directions to give a clear genetic structure for BR resistance and apply in resistance breeding. Furthermore, with increasingly more races and variabilities being discovered around the world (Singh et al. 2011; Rouhrazi and Khodakaramian 2014; Burlakoti et al. 2018), more resistance resources are becoming an urgent need.

Table 3.3 BR genes/QTLs identified in the genus *Brassica*

Population origins	Population type	Race	Results	References
Broccoli × cabbage	F_2, F_3	–	Identified two major QTLs on LG1 and LG9 and two additional QTLs	Camargo et al.(1995)
B. napus	DH	4	*Xca4* was positioned on LG N5 of A genome	Vicente et al. (2002)
Chinese cabbage	F_2	1 and 4	Identified two QTLs for race 1 resistance on chromosome A06 and four QTLs for race 4 resistance on chromosome A02, A06, and A09	Soengas et al. (2007)
Broccoli × cabbage	F_2, F_3	–	Identified two major QTLs on LG2 and LG9, and two minor ones	Doullah et al. (2011)
Cabbage × broccoli	F_2	1	Identified one major QTL on chromosome C2 and two minor ones	Kifuji et al. (2013)
Broccoli × cabbage	F_2, F_3	1	Identified one major QTL on chromosome C8 and two minor ones	Tonu et al. (2013)
Cauliflower	F_2	–	The major locus *Xca1bo* was mapped in 1.6-cM interval on chromosome C3	Saha et al. (2014)
Cabbage	F_2, F_3	–	Identified one major QTL on chromosome C3 and three minor ones	Lee et al. (2015)
Ethiopian mustard	F_2	1	The resistance locus *Xca1bc* was mapped in a 6.6 cM interval on chromosome B7	Sharma et al. (2016)

3.6.3 *Fusarium Wilt (FW)*

Fusarium yellows or Fusarium wilt (FW) is one of the important diseases in the world and was first found in the USA and then in Japan and China (Xing et al. 2016). FW is caused by soilborne fungus, *F. oxysporum,* and contains many varieties and infects major crops and vegetables such as tomato, cotton, melon, and banana (Ploetz 2006; Ulloa et al. 2006; Charoenporn et al. 2010; Oumouloud et al. 2013). In Brassicaceae, leaf vegetables including cabbage (*B. oleracea*), Chinese cabbage, pak choi, komatsuna, and turnip (*B. rapa*) are usually infected by *F. oxysporum*. These *Brassica* vegetables are major a food source in Asia, especially in Japan, China, and Korea, which is threatened by the FW disease. Two forma specialis of *F. oxysporum* can inoculate Brassicaceae; *F. oxysporum* f. sp. *conglutinans* (*Foc*) inoculates *B. oleracea* and *B. rapa*, and has higher virulence on *B. oleracea* than on *B. rapa*, while *F. oxysporum* f. sp. *rapae* can inoculate only *B. rapa* (Daly and Tomkins 1995; Enya et al. 2008). When Brassicaceae is inoculated by *F. oxysporum*, the parenchyma tissue between the veins of their leaves becomes yellow and yellowing spots spread to the whole leaves (Walker 1930; Sherf and MacNab 1986; Daly and Tomkins 1995). The plants also show defoliation and stunted growth. Simultaneously or after the loss of the normal green color in the infected plants, the vascular elements in the diseased tissue become brown and the plants will finally die.

The pathogen usually invades the plants through young root, but can also invade through wounds in older roots (Sherf and MacNab 1986; Daly and Tomkins 1995). They move via water-conducting xylem tissue to root, stem, and leaves. In the susceptible cultivar, conidia attach to the root hair and the emergence site of the lateral roots, and grow into the central root surfaces between 1 to 3 days post-inoculation (dpi) (Pu et al. 2016). From 4 to 6 dpi, the mycelia spread from the epidermis into the cortical tissues, enter the xylem vessels, and move upward. After 7 dpi, hyphae are observed not only in the root, but also in the neighboring parenchymal tissues and surrounding cortical tissues. Microscopic analysis compared between the root of the resistant and susceptible cabbage cultivars after *Foc* inoculation showed that the infected points were observed in the susceptible cultivar at 1 dpi but not in the resistant cultivar (Pu et al. 2016). From 3 to 12 dpi, the infected points increase further in the susceptible cultivar. In the resistant cultivar, the infected points were observed from 3 dpi, but the number of points did not increase further and is less than the susceptible cultivar. Li et al. (2015b) also performed microscopic analysis using the root of the resistant and susceptible cabbage cultivars. In this report, there are few differences between resistant and susceptible cultivars from 1 to 3 dpi, while from 4 to 6 dpi, colonization was observed in the susceptible cultivar, but not in the resistant cultivar. They also compared the colonization pattern in root, stem base, upper stem, and petiole between the resistant and susceptible cultivars. In the susceptible cultivar, the colonization was observed in root, stem base, upper stem, and petiole, while in the resistant cultivar, few fungi were observed in root and stem base, and not in the upper stem and petiole. In summary, the fungus developmental speed is slower in the

resistant cultivar than in the susceptible cultivar, and the resistant cultivar restricts the fungus development and spreading.

Only two types of *Foc* resistance have been reported: type A and B. Type A resistance, which is stable under high or low temperature, followed a single dominant inheritance pattern and has been proven to be very effective in resistance breeding and has been introduced into various cabbage cultivars (Walker 1930, 1933; Blank 1937), generating the first series of resistant cabbage cultivars, distributed in 1920s, including "Wisconsin Hollander" (winter/storage type), "Wisconsin All Seasons" (mid-season type), "Copenhagen Market" (mid-season type), "All Head Early" (flat-head type), etc. (Walker et al. 1927; Walker and Blank 1934). At the same time, type B resistance is unstable under high temperature (above 24 °C) and follows a polygenic inheritance pattern, limiting its use in breeding (Walker et al. 1927; Walker 1930; Farnham et al. 2001). *Foc* race 1 has been found worldwide, while race 2 has only been reported in USA and Russia (Bosland et al. 1988; Morrison et al. 1994). While type A resistance is very effective to race 1, the cultivars of type A resistance have successfully controlled FW for decades. However, type A major gene resistance can be overcome by *Foc* race 2 and the genetic control of host resistance to race 2 remains unclear. Thus, efforts are needed to clarify the genetic structure of cabbage resistance to race 2. *Foc* favors hot climate and plentiful rainfall and can survive for more than 10 years even without a host, making it difficult to control through traditional methods like seed treatment, rotation, and fungicide (Tisdale 1923; Bosland et al. 1988; Fravel et al. 2003). Once this disease is present in the field, using FW resistant cultivar is the only successful method to maintain the yield.

Resistance genes to FW have been isolated in *A. thaliana*, *B. rapa*, and *B. oleracea*. In *A. thaliana*, six dominant RESISTANCE TO FUSARIUM OXYSPORUM loci (*RFO1-6*) contribute to the resistance to *F. oxysporum* f. sp. *matthioli* (Diener and Ausubel 2005). The strongest locus encodes *RESISTANCE TO FUSARIUM OXYSPORUM 1* (*RFO1*), identical to *WALL-ASSOCIATED KINASE-LIKE KINASE 22* (*WAKL22*), which encodes for receptor-like kinase. *RFO1* does not have LRR domain, making *RFO1* an atypical type resistance gene. RFO1 interacts with RFO2, RFO4, and RFO6, and RFO2 is identified as the receptor-like protein gene, which is a homologue to the PSY1 peptide receptor gene, *PSY1R* (Shen and Diener 2013). RFO3 and RFO5 are independent of RFO1, and *RFO3* encodes a receptor-like kinase (Cole and Diener 2013; Diener 2013). To *Foc* race 1, RFO7 is associated with the resistance in *A. thaliana* (Diener 2013). In *B. rapa*, inoculation test using F_2 population showed that a single dominant gene regulates the resistance to *Foc* (Shimizu et al. 2014). The neighbor genes, Bra012688 and Bra012689, were identified in FW resistance inbred line of Chinese cabbage by transcriptome analysis (Shimizu et al. 2014). These two genes have TIR, NB, and LRR domains. In the susceptible line, these two genes are completely deleted. Shimizu et al. (2014) did not conclude whether Bra012688 or Bra012689 is the resistance gene to FW because all FW resistance lines contained these two genes and all susceptible lines lacked these two genes. In *B. oleracea*, the type A single dominant resistance gene *FOC1* has been studied extensively in recent years, which is favored greatly by the release of the reference genome (Liu et al. 2014). Pu et al. (2012) mapped FW resistance gene *FocBo1* to LG seven (O7)

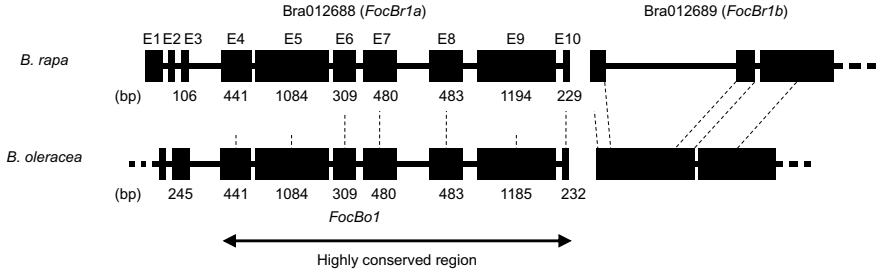

Fig. 3.35 Genetic region containing resistance genes to Fusarium wilt (FW) conserved in *B. rapa* and *B. oleracea*. Black boxes and bars show exons and introns, respectively. Numbers under boxes show each length

using both BSA and QTL analyses in cabbage. Lv et al. (2013) constructed a linkage map based on a cabbage DH population. Lv et al. (2014a) mapped the gene to the interval between two InDel markers, M10 and A1, flanking the resistance gene at 1.2 and 0.6 cM, respectively, and used these markers to breed resistant hybrids. Lv et al. (2014b) ultimately mapped the candidate resistance gene *FOC1* using an enlarged cabbage F_2 population to a re-predicted Bol037156, which encodes a putative TIR-NB-LRR type R protein. Shimizu et al. (2015) further mapped the resistance locus *FocBo1* by using 139 recombinant F_2 plants derived from resistant cabbage AnjuP01 and susceptible broccoli GCP04, and identified an orthologous gene of Bra012688 as a candidate gene. The genetic region including the FW resistance genes is conserved between *B. rapa* and *B. oleracea*. Most of the exons of Bra012688, one of the candidates to FW resistance gene in *B. rapa*, are conserved in *FocBo1*, but Bra012689 is not conserved in *B. oleracea*, indicating that Bra012688 may be the resistance gene to FW in *B. rapa* (Fig. 3.35).

Plant pathogens are categorized into biotrophs and necrotrophs by their lifestyles (Glazebrook 2005). Biotrophic pathogens get nutrients from living host tissues, while necrotrophic pathogens kill host tissue and gain nutrients from dead tissues. SA, jasmonic acid (JA), and ethylene (ET) are the phytohormones related to disease resistance. SA-dependent defenses act against biotrophic pathogens, and JA- and ET-depending defenses act against necrotrophic pathogens. *F. oxysporum* is considered a hemibiotrophic disease, because it begins its infection cycle as a biotroph, but change to a necrotroph at the later stage (Lyons et al. 2015). Transcriptome analysis using RNA-seq gives expression levels of all genes, allele-specific expression, and splicing variants (Mortazavi et al. 2008). Fusarium-inoculated and mock-treated plants were compared with each other using transcriptome analysis in *A. thaliana*, *B. rapa*, and *B. oleracea*. In *B. rapa*, using FW resistant and susceptible inbred lines of Chinese cabbage, the differentially expressed genes (DEGs) were identified by comparison between with and without *Foc* inoculation (Miyaji et al. 2017). Gene Ontology (GO) analysis using upregulated DEGs at 24 h after inoculation suggested that the resistant lines activated systemic acquired resistance, and that the susceptible lines activated tryptophan biosynthetic process and responses to chitin and ET

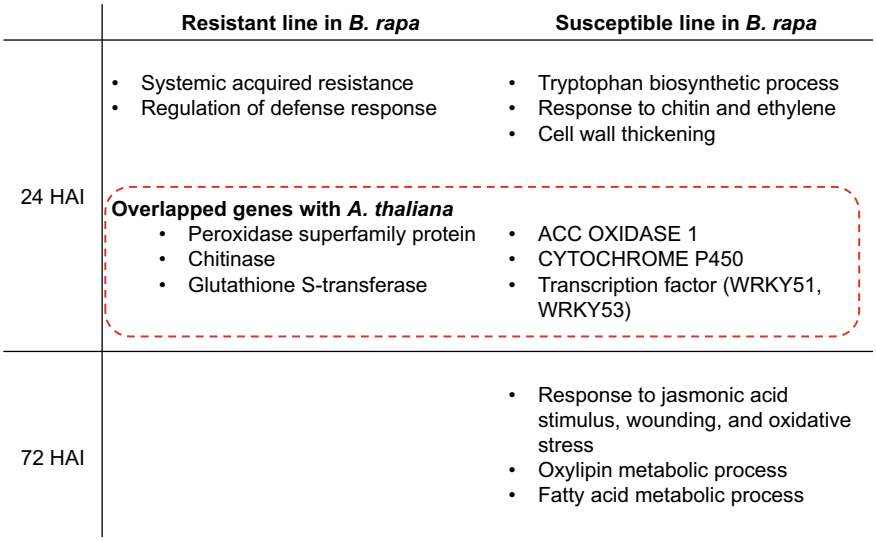

Fig. 3.36 Transcriptional change after *Foc* inoculation in the resistant and susceptible lines in *B. rapa*. HAI; hours after inoculation

(Fig. 3.36). At 72 h after inoculation, GO analysis indicated that the genes related to response to biotic stimulus and response to stress were expressed in the susceptible lines, but there are no overrepresentation in the resistant lines (Fig. 3.36). The transcriptome analysis in *A. thaliana* after *F. oxysporum* infection was also reported (Zhu et al. 2013); DEGs were compared between *B. rapa* and *A. thaliana* at the same time point, 24 h after inoculation (Miyaji et al. 2017). Genes encoding the peroxidase superfamily protein, chitinase, glutathione S-transferase, ACC OXIDASE 1, CYTOCHROME P450, and some TFs including WRKY51 and WRKY53 were common with between *A. thaliana* Columbia-0 (medium susceptible), and the resistant and susceptible inbred lines of Chinese cabbage (Fig. 3.36). In *B. oleracea*, RNA-seq was performed in the resistant cabbage variety and DEGs were identified by comparison between the samples inoculated with *Foc* or distilled water (Xing et al. 2016). They performed GO, clusters of orthologous groups (KOG), and Kyoto encyclopedia of genes and genomes pathway database (KEGG) analysis. From these analysis, calcium signaling, mitogen-activated protein kinase (MAPK) signaling, SA-mediated hypersensitive response, SA-dependent systemic acquired resistance, JA- and ET-mediated pathways, and the lignin biosynthesis pathway were activated at the early time point after *Foc* inoculation, indicating that their signaling and pathways are important for *Foc* resistance in cabbage (Fig. 3.37).

Xylem sap proteome of the non-inoculated and *Foc*-inoculated root was also performed using liquid chromatography–mass spectrometry (LC-MS/MS) in the resistant and susceptible cultivars in *B. oleracea* (Pu et al. 2016). A large portion of up- and downregulated proteins was categorized into the protein acting on carbohydrates in the resistant and susceptible cultivars, suggesting that these proteins may have a

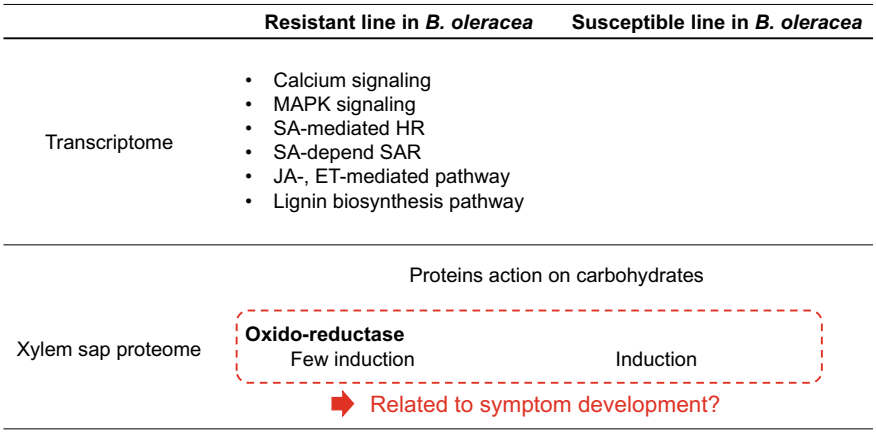

Fig. 3.37 Transcriptional change and protein concentration after *Foc* inoculation in the resistant and susceptible lines in *B. oleracea*. SA; salicylic acid, HR; hypersensitive resistance, SAR; systemic acquired resistance, JA; jasmonic acid, ET; ethylene

role for *Foc* resistance. Both up- and downregulated oxidoreductases were induced in the susceptible cultivar, while there were only a few inductions of oxidoreductases in the resistant cultivar, indicating that the induced oxidoreductases are related to symptoms development in the susceptible cultivar. To note, they identified ten *Foc* cysteine-containing secreted small proteins as candidate effectors. Proteome was also performed using two races of *Foc* that differ in pathogenicity, race 1 and 2 (Li et al. 2015a). Race 2 has stronger pathogenicity compared with race 1. The high abundance proteins contained carbohydrate, amino acid, and ion metabolism in race 2, indicating that these proteins may be involved in the race 2's stronger pathogenicity. *Foc* has four isoforms of the homolog of secreted-in-xylem 1 (SIX1) protein, and a SIX1 homolog is required for the full level of virulence on cabbage (Li et al. 2016a). They also analyzed whether SIX1 works as an avirulence gene in *Foc* by inoculation test using the FW resistance cabbage variety. Cabbage showed no disease symptoms by the inoculation of both *Foc* with WT and mutational *SIX1*, indicating that *SIX1* is not an avirulence gene, but a virulence gene in *Foc*.

3.6.4 Clubroot (CR)

Clubroot (CR) disease, caused by the soilborne pathogen *P. brassicae* (*Pb*), is now threatening almost all the *Brassica* crops worldwide. CR was firstly reported in Russia in 1878, and the disease rapidly expanded to Europe, Asia, and USA during nineteenth and early twentieth century, becoming one of the most serious problems in almost every *Brassica* production area around the world. *Pb* infection is a two-phase process. The primary phase occurs in root hairs, and the secondary phase occurs

in cells of the cortex and stele of the root. During the latter phase, multinucleate plasmodia induce clubs on roots, inhibiting the nutrient and water transport, causing abnormal cell enlargement, and uncontrolled cell division of infected roots, thus deforming them with characteristic clubs. Thus, the quality and commercial value of the crop products are seriously compromised (Piao et al. 2009). *Pb* has a wide host range and can affect cruciferous plants including all *Brassica* crops, common weeds like charlock and *A. thaliana*, as well as some non-cruciferous plant species such as *Tropaeolum majus* and *Reseda alba* (Dixon 1980; Ludwig-Müller et al. 1999). *Pb* has a complex pathotype differentiation and has been extensively studied (Ayers 1957). Currently, there are two main systems used for classification: the Williams system (four differential hosts) and the European clubroot differential (ECD) set (15 differential hosts), proposed by Buczacki et al. (1975), both of which are widely used in pathotype identifications all over the world (Donald et al. 2006). *Pb* is favored at low pH, and wet and warm weather (Diederichsen et al. 2009). The pathogen variation and its ability to survive in soil as resting spores for up to 15 years make it difficult to control by cultural practices or chemical treatments (Dixon 1980; Voorrips 1995; Kageyama and Asano 2009). Thus, breeding of resistant cultivars is a desirable means of utilizing the host resistance and reducing pollution to the environment. Currently, several important CR resistance genes/QTLs have been mapped or cloned, and MAS and introgression breeding have been widely used in improving the resistance in the *Brassica* crops (Manzanares-Dauleux et al. 2000; Nomura et al. 2005; Ueno et al. 2012; Lee et al. 2016; Li et al. 2016d; Hatakeyama et al. 2017).

Extensive studies for inheritance analysis have been performed in *Brassica* crops including *B. rapa*, *B. oleracea,* and *B. napus*. In *B. rapa*, many studies have indicated major dominant genes, which conferred resistance to specific *Pb* pathotypes (Wit and Van De Weg 1964; Toxopeus and Janssen 1975). A few important turnip resistance sources have been used for genetic analysis, resistance gene mapping, and *B. rapa* breeding. The major resistance from European fodder turnip cultivar "Siloga" was proved and widely used in turnip and Chinese cabbage breeding (Kuginuki et al. 1997; Suwabe et al. 2003, 2006; Hatakeyama et al. 2013). Other turnips possessing *CR* resistance genes include inbred line N-WMR-3 carrying major gene *Crr3*, "Gelria R" carrying major dominant resistance to race 4, European fodder turnip "Debra" carrying major genes *CRk* and *CRc*, and inbred line ECD04 with quantitative resistance to a series of *Pb* isolates, which was revealed by genetic analysis using different F_2, F_3, and BC segregation populations (Piao et al. 2004; Hirai et al. 2004; Saito et al. 2006; Sakamoto et al. 2008; Chen et al. 2013). Other major dominant genes were identified in Chinese cabbage accessions including T136-8 with *CRa* to race 2 (Matsumoto et al. 1998; Ueno et al. 2012), "Akiriso" and "CR Shinki" with *CRb* to race 3 and 4 (Kato et al. 2012, 2013; Zhang et al. 2014), "Jazz" with resistance gene *Rcr2* to multi pathotypes (Huang et al. 2017), and 85-74 with race 4 resistance *CRd* (Pang et al. 2018). Quantitative-inherited resistance to a few pathotypes was found in T19 (Yu et al. 2017). Also, a pak choi cultivar "Flower Nabana" was found with pathotype 3 major dominant resistance gene *Rcr1* (Chu et al. 2014; Yu et al. 2016).

In *B. oleracea*, most of these studies concluded that inheritance of this trait was polygenic (Piao et al. 2009). Using cabbage segregation populations, the resistance was shown to be recessive and controlled by two genes with additive effects (Chiang and Crête 1976). Also, dominant or incomplete dominant inheritance was found in cabbage and kale (Hansen 1989; Laurens and Thomas 1993). Further, based on qualitative and quantitative analyses, Voorrips and Kanne (1997) suggested four types of inheritance, one of which was controlled by two complementary genes. The polygenic inheritance of CR resistance in *B. oleracea* was further validated in broccoli accession CR7 (Figdore et al. 1993), cabbage resources Bindsachsener, Anju, C1220, and GZ87 (Voorrips et al. 1997; Nagaoka et al. 2010; Lee et al. 2016; Peng et al. 2018), and kale cultivars C10 and K269 (Grandclément and Thomas 1996; Moriguchi et al. 1999; Rocherieux et al. 2004; Nomura et al. 2005).

In *B. rapa*, several *CR* genes/QTLs conferring complete resistant accessions against specific pathogen isolates were found, and more than ten loci have been identified (Table 3.4). The mapping and cloning of the first loci *CRb*/*CRa* took over 20 years. Matsumoto et al. (1998) firstly mapped the dominant major gene *CRa* in ECD02 on LG3. Ueno et al. (2012) fine mapped the *CRa* locus using synteny to the *A. thaliana* genome and revealed a candidate gene encoding a TIR-NBS-LRR protein. This was the first report on the molecular characterization of a *CR* resistance gene in the genus *Brassica*. Then, an *R* locus to pathotype 4, *CRb*, was mapped by Piao et al. (2004) to an interval of 3 cM from the Chinese cabbage cultivar "CR Shinki". Kato et al. (2012) identified of a *CR* resistance locus CRb^{Kato} to pathotype group 3 in Chinese cabbage "Akiriso", and the markers were also linked to *CRb*. To fine map *CRb*, Kato et al. (2013) further developed 28 markers and located *CRb* in the 140-kb genomic region and found candidate resistance genes. Zhang et al. (2014) narrowed *CRb* locus to a region of 83.5 kb on a BAC clone, with several candidates. *CRb* was tightly linked to *CRa* and CRb^{Kato}. To identify the relationship, Hatakeyama et al. (2017) determined the sequence of an approximately 64-kb region, and CRb^{Kato} and *CRa* were determined to be the same TIR-NB-LRR gene, while *CRb* might be a different but closely linked locus. Another example is *Crr1-4*. At first, Kuginuki et al. (1997) employed RAPD marked to study *CR* resistance gene *Crr1* in turnip cultivar "Siloga" using a DH population. Suwabe et al. (2003) identified *Crr1* and *Crr2* from G004 (Siloga derived) and concluded that these two loci were complementary. Besides, a weak QTL *Crr4* was detected (Suwabe et al. 2006). Hirai et al. (2004) identified and mapped a novel locus *Crr3* using RAPD markers, which originated from the turnip cultivar "Milan White". Saito et al. (2006) used Chinese cabbage progenies and mapped the *Crr3* gene in a 0.35 cM segment. Sakamoto et al. (2008) developed populations derived from resistant turnip cultivar "Debra" and identified two *CR* loci, *CRk* and *CRc*. *CRk* was located close to *Crr3*. Through fine mapping, Hatakeyama et al. (2013) revealed that *Crr1* comprises two loci: *Crr1a* and *Crr1b*. *Crr1a* was cloned from the resistant line G004, encoding TIR-NB-LRR, and was functionally confirmed in susceptible *A. thaliana* and *B. rapa*. With the development of genomic and molecular genetics, especially the release of the reference genome sequence of *B. rapa*, more *R* loci were discovered. Chen et al. (2013) used SSR markers to map the resistance in ECD04, and six QTLs were identified. Chu et al.

Table 3.4 Mapped *CR* genes/QTLs in *B. rapa*

Resistance source	Species	Populations	Race/Isolate	Markers/Techniques	Important loci/genes	References
T136-8	Chinese cabbage	F_2	Race 2	RFLP, STS	*CRa* on A03	Matsumoto et al. (1998)
G004 (Siloga derived)	Turnip	F_2	Race 2 and others	SSR	*Crr1* on A08	Kuginuki et al. (1997), Suwabe et al. (2003, 2006)
G004 (Siloga derived)	Turnip	F_2	Race 2 and others	SSR	*Crr2* on A01	Suwabe et al. (2003, 2006)
N-WMR-3 (Milan White derived)	Turnip	F_2, F_3, F_4	Race 2	RAPD	*Crr3* on A03	Hirai et al. (2004)
N-WMR-3 (Milan White derived)	Turnip	F_2, F_3, F_4	Race 2	STS	*Crr3* in a 0.35 cM segment on A03	Saito et al. (2006)
Gelria R	Turnip	F_2	Race 4	SCAR	*CRb* on A03	Piao et al. (2004)
Siloga	Turnip	F_2	Race 2 and others	RFLP	*Crr4* on A06	Suwabe et al. (2006)
Debra	Turnip	F_2	Race 2 and others	STS, AFLP	*CRk* on A03, and *CRc* on A02	Sakamoto et al. (2008)
Akiriso	Chinese cabbage	F_2	Race 3	SSR, CAPS	CRb^{Kato}	Kato et al. (2012)
T136-8	Chinese cabbage	F_2	Race 2	Mutation analysis	*CRa* encodes TIR-NB-LRR protein	Ueno et al. (2012)
ECD04	Turnip	BC	Pb2, Pb4, Pb7, and Pb10	SSR, UGMS	Six QTLs on A01, A03, and A08	Chen et al. (2013)
CR Shinki	Chinese cabbage	F_2	Race 3	SSR	CRb^{Kato}, 140 kb interval on A03	Kato et al. (2013)
G004 (Siloga derived)	Turnip	F_2	Race 2 and others	Functional analysis	*Crr1a* encodes TIR-NB-LRR protein	Hatakeyama et al. (2013)
FN	Pak choi	F_2	Pathotype 3	SSR, RNA-seq	*Rcr1*, 240 kb interval on A03	Chu et al. (2014)
CR Shinki	Chinese cabbage	F_2	Pathotype 4	BSA, BAC	*CRb*, 83.5 kb interval on A03, with two candidates	Zhang et al. (2014)

(continued)

Table 3.4 (continued)

Resistance source	Species	Populations	Race/Isolate	Markers/Techniques	Important loci/genes	References
Flower Nabana	Pak choi	F_2	Pathotype 3	KASP, BSR-seq	*Rcr1* on A03, with two candidates	Yu et al. (2016)
T19	Chinese cabbage	BC	Pathotypes 2, 3, 5, 6 and 8	SNP, GBS	*Rcr4* on A03, Rcr8 on A02, and Rcr9 on A08	Yu et al. (2017)
Jazz	Chinese cabbage	F_2	Pathotypes 2, 3, 5, 6, and 8	KASP, BSR-seq	*Rcr2* on A03, with two candidates	Huang et al. (2017)
CR Shinki	Chinese cabbage	F_2, F_3	Pathotype group 3	Functional analysis	*CRa* and CRb^{Kato} are the same TIR-NB-LRR allele	Hatakeyama et al. (2017)
20-2ccl	Chinese cabbage	BC	–	RAPD, SSR	*CrrA5* on A05	Nguyen et al. (2018)
85-74	Chinese cabbage	F_2	Race 4	BSA-Seq	*CRd*, 60 kb interval on A03	Pang et al. (2018)

(2014) mapped a *CR* gene from pak choi cultivar "Flower Nabana" to the region between 24.26 Mb and 24.50 Mb on LG A03. Yu et al. (2016) applied bulked segregant analysis sequencing (BSA-seq) and identified a novel resistance gene *Rcr1*, and Bra019409 and Bra019410 encoding TIR-NB-LRRs were probable candidates. Yu et al. (2017) performed genotyping-by-sequencing (GBS) and revealed three QTLs for CR resistance to six pathotypes. A single co-localized QTL, designated as *Rcr4,* was on chromosome A03. Two QTLs for resistance to a novel pathotype 5x, designated *Rcr8* and *Rcr9*, were detected, respectively. Huang et al. (2017) adopted SNP-based competitive allele-specific PCR (KASP) markers and bulked segregant RNA-sequencing (BSR-seq) strategies to identify the locus *Rcr2* in CR-resistant Chinese cabbage "Jazz", and *Rcr2* was fine mapped to a 0.4 cM interval, with two TIR-NBS-LRRs as the likely candidates. Nguyen et al. (2018) found a dominant monogenic resistance locus *CrrA5* in a Chinese cabbage inbred line 20-2ccl on the LG 5. Using BSA-seq, Pang et al. (2018) identified a new locus *CRd* to a 60 kb region on chromosome A03, which was located upstream of *Crr3*, using an F_2 segregation population derived from the resistant line 85-74.

Using omics techniques such as RNA-seq and proteomics, significantly related genes were found to be involved in plant–pathogen interaction, calcium ion influx, pathogenesis-related (PR) pathway, chitin metabolism, hormone signaling, cell-wall modifications, antioxidant protein expression, glucosinolate biosynthesis, and glycolysis metabolism (Cao et al. 2008; Verma et al. 2014; Chen et al. 2016; Song et al. 2016a; Xu et al. 2016). Also, plant hormones, especially SA and JA, were all believed to be important in the interactions (Lovelock et al. 2013; Chu et al. 2014; Zhang et al. 2016b; Manoharan et al. 2016; Jia et al. 2017; Luo et al. 2018). Based on these

data, some important gene families were further studied for their possible roles during *Brassica–Pb* interaction. MAPK cascades play key roles in responses to various biotic stresses. Piao et al. (2018) found 5 *BraMKK* and 16 *BraMPK* genes that exhibited a significantly different expression pattern between a pair of CR-resistant and susceptible near-isogenic lines (NILs). *SWEET* genes have been demonstrated as the targets of extracellular pathogens. Li et al. (2018a) identified several *BrSWEET*s that were significantly upregulated, especially in CR susceptible NIL upon *Pb* infection. Chitinases are believed to function as a guardian against chitin-containing pathogens. Chen et al. (2018) revealed that 14 chitinase genes were expressed differentially in response to *Pb* between CR resistance and susceptible NILs. Furthermore, reduced pathogen DNA content and CR symptoms were observed in the CR susceptible NILs after their treatment with chitin oligosaccharides 24 h prior to inoculation with *Pb*. The findings indicate that chitinases play a crucial role in pathogen resistance of the host plants.

Resistance in *B. oleracea* appears to be determined by quantitative genes (Piao et al. 2009). So far, a few CR QTLs were identified in cabbage, kale, and broccoli. Figdore et al. (1993) first identified three QTLs showing resistance to race 7 using broccoli. In resistant kale line C10, Grandclément and Thomas (1996) performed QTL detection with RAPD markers, suggesting the existence of at least two genetic mechanisms in the resistance; Rocherieux et al. (2004) further found two to five QTLs depending on the five pathotype used. Of the nine QTLs fully identified, *PbBo1* was detected in all isolates and explained 20.7–80.7% of the phenotypic variation. Using another resistant kale line K269, Moriguchi et al. (1999) constructed a genetic map and identified two QTLs for resistance; similarly, Nomura et al. (2005) identified three QTLs. In cabbage, Voorrips et al. (1997) firstly reported two QTLs, *pb-3* and *pb-4*, and a minor QTL contained in landrace Bindsachsener. Nagaoka et al. (2010) identified a major QTL, *PbBo(Anju)1* on LG 2, from cabbage accession Anju with a maximum LOD score of 13.7. Tomita et al. (2013) examined the major locus *PbBo(Anju)1* and other QTLs and found that a single major locus was not enough to confer sufficient resistance. Lee et al. (2016) employed the GBS technique to construct a high-resolution genetic map. QTLs survey using $F_{2:3}$ progenies revealed two and single major QTLs for race 2 and race 9, respectively. The QTLs showed similar locations to the previously reported *CR* loci for race 4 in *B. oleracea,* but were in different positions from any of the *CR* loci found in *B. rapa*, indicating the divergence of resistance genes in A and C genome. Peng et al. (2018) performed QTL analysis with SNP microarray and identified 23 QTLs for disease incidence and the other two correlated traits, individually explaining 6.1–17.8% of the phenotypic variation. In summary, over 30 QTLs have been found in *B. oleracea* so far (Table 3.5), indicating the complex genetic basis of CR resistance. It is difficult to compare these QTLs, due to the use of different CR sources and isolates.

Table 3.5 Mapped *CR* genes/QTLs in *B. oleracea*

Resistance source	Species	Population type	Race/Isolate	Technique	Mapping results	References
CR7	Broccoli	F_2	Race 7	RFLP	Three QTLs on LG1, LG4, and LG9	Figdore et al. (1993)
C10	Kale	F_2	ECD 16/31//31	RAPD	At least two QTLs	Grandclement and Thomas (1996)
Bindsachsener	Cabbage	DH	Field isolate	RFLP, AFLP	Two QTLs: *pb-3* on LG3 and *pb-4* on LG1	Voorrips et al. (1997)
K269	Kale	F_2	Race 1, 3	RAPD, AFLP	One QTL on LG3	Moriguchi et al. (1999)
C10	Kale	F_2	P1, P2, P4, P7	RAPD, RFLP, ACGM	Nine QTLs on LG1, LG2, LG3, LG4, LG5, LG8, and LG9	Rocherieux et al. (2004)
K269	Kale	F_2	Three field isolates	SCAR	Three QTLs: QTL1 on LG1, QTL3 on LG3, and QTL9 on LG9	Nomura et al. (2005)
Anju	Cabbage	F_2, F_3	Race 4	SSR, SRAP, SCAR	Five QTLs on LG O2, O3, and O7; Major one is *PbBo(Anju)1* on O2	Nagaoka et al. (2010)
C1220	Cabbage		Race 2, 9	GBS	Three QTLs on chromosome C2 and C3	Lee et al. (2016)
GZ87	Cabbage	F_2	Race 4	SNP Microarray	23 QTLs	Peng et al. (2018)

3.6.5 Marker-Assisted Selection (MAS)

Molecular markers are specific inheritable and detectable DNA segments, which can be used for linkage map construction, gene mapping, and MAS. The marker types and mapping methods have been improved greatly from 1990s till now in the genomic era. In 1990s, low-efficiency RAPD, AFLP, CAPS/RFLP markers were mainly used. Since 2000s, convenient and easily detectable SSR, microsatellite, and InDel markers were applied in identification of the resistance genes. From 2010 onward, high-throughput-based methods of mapping have become popular, such as

SNP-based markers like KASP markers, microarray, BSA, and GWAS. For example, Huang et al. (2017) adopted KASP markers and BSA-seq strategies to rapidly identify the locus *Rcr2* in CR-resistant Chinese cabbage cultivar "Jazz", and *Rcr2* was fine mapped to a 0.4 cM interval, with two TIR-NB-LRRs as the candidates. Of special note, KASP technology possesses high levels of assay robustness and accuracy with notable savings in cost and time. For example, Li et al. (2016b) developed a KASP marker based on the TuMV resistance gene *retr02*, which could accurately genotype the allele in Chinese cabbage accessions.

MAS is a useful method to predict the phenotype at early developmental stages without field trials. Nowadays, there are some DNA markers against FW or CR resistance genes in the *Brassica* vegetables (Kawamura et al. 2015, 2017). The genomic era is also symbolized with high-efficiency integrated breeding (HIB), in which multi-MAS methods such as foreground and background analysis are combined with traditional methods such as microspore culture and backcrossing. For example, in the study of Liu et al. (2017b), the resistance-specific markers as well as genome background markers were used in cabbage resistance breeding to FW. Combined with microspore culture and backcrossing, the authors presented a rapid and effective way of generating FW resistance introgression lines in BC_2 generation. During HIB, the genomic background analysis is of great help in eliminating the undesirable linkage drags and rapidly finding the desirable individual.

New tools like CRISPR/Cas9-based genome editing provide new approach of molecular design breeding (MDB) (Cong et al. 2013; Li et al. 2013a). Compared with traditional genetic modification technologies such as zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), the RNA-guided Cas9 system is highly efficient and flexible (Nekrasov et al. 2013; Shan et al. 2013a). This technique has been widely used in field crops including rice, wheat, maize, and cotton, and model plants such as *A. thaliana* and tobacco (Altpeter et al. 2016; Scheben et al. 2017). Lawrenson et al. (2015) firstly employed CRISPR/Cas9 system on *B. oleracea* by targeting multicopy genes *BolC.GA4.a*, leading to Cas9-induced mutations and an expected dwarf phenotype associated with knockout of the target genes. Also, in *B. napus*, successful editing of target genes including *CLAVATA* and *FAD2* has resulted in inheritable and stable mutations and desirable phenotypes (Yang et al. 2017b; Okuzaki et al. 2018), displaying great potential in its application. With increasingly more genome and transcriptome sequences being available, CRISPR/Cas9 technique will, with no doubt, bring revolution in crop breeding as a fast and accurate method.

3.6.6 Perspective

For certain *Brassica* species, the resistance resources to some diseases such as BR and CR are very limited. Generally, A genome is rich in TuMV and CR resistance and B genome possesses BR resistances. The inter-species crossing within *Brassica* genus are widely adopted, using embryo rescue, reciprocal crossing, and MAS, and inter-species hybridizations have been used to transfer and utilize the resistance.

Fortunately, the six basic species and others such as *B. incana*, *B. cretica*, and *B. fruticulosa* in the *Brassica* genus, as well as its close Brassicaceae relatives such as *Erucastrum cardaminoides*, *Raphanus raphanistrum*, and *Sinapsis arvensis* could be used for crossing to facilitate resistance gene exchanges in breeding programs.

A sole resistance gene is easily broken down, which is caused by the pathogen variations as well as global climate changes. For example, a few *B. rapa*, *B. oleracea*, and *B. napus* varieties have been successfully cultivated, with resistance to the specific CR pathogen *Pb* races (Rocherieux et al. 2004; Werner et al. 2008; Chen et al. 2013). However, they all exhibited loss of resistance within a few years (Kuginuki et al. 1999). At the same time, the vast genetic variability of the CR pathogen *Pb* and infection by multiple races have been reported (Buczacki et al. 1975; Kuginuki et al. 1999). For BR pathogen *Xcc*, pathogen variations were frequently discovered and at least 11 races have been reported (Singh et al. 2011; Rouhrazi and Khodakaramian 2014; Burlakoti et al. 2018). Undoubtedly, more durable resistance is in urgent need to secure the *Brassica* crops production. Durable resistance was first defined by Johnson (1984) as resistance that remains effective during its prolonged and widespread use in an environment favorable to the disease. Complete race-specific *R* gene is highly effective but is easily broken down; polygenic quantitative resistance is considered to be more durable than qualitative resistance, but its effectiveness varies between cropping seasons due to environmental conditions (Lindhout 2002). Thus, the pyramiding of qualitative resistance with a high level of quantitative resistance in cultivars is an ideal way to maximize the effectiveness and durability of the resistance. This pyramiding model is supported and used in resistance breeding to BR (Vicente et al. 2002) and CR (Piao et al. 2009; Tomita et al. 2013). Thus, combining quantitative resistances with sole *R* genes is a promising strategy in resistance breeding.

3.7 Abiotic Stress

Plants are sessile organisms. Therefore, the environmental conditions where a plant is cultivated must be tolerable to ensure their successful growth. Abiotic conditions such as temperature, photoperiodicity, moisture, salinity, and soil conditions (nutritional content, pH, and physical characteristics such as porosity) all impose selective pressures upon plants. The adaptations of plant species to various abiotic stressors are thus dependent upon the climatic conditions present within their habitat.

Vernalization is an adaptation that ensures the plant flowers during the spring, when seasonal conditions are amenable to reproductive success (Shea et al. 2018a). Other abiotic stresses such as high ambient temperatures (those found in tropical and subtropical climates) and salt tolerance are also additional limiting factors of agronomic importance in the successful cultivation of the *Brassica* vegetables. As such, researches examining the molecular mechanisms involved in drought, heat, and salt response have been conducted to identify the regulatory pathways and genes involved.

Many genes induced by abiotic stress are upregulated irrespective of the type of abiotic stress, suggesting a shared regulatory pathway in stress response of plants. As such, the genes associated with abiotic stress response are classified into three groups. The first group of genes encodes for proteins that act to protect plant cells against stresses, e.g., late-embryogenesis abundant proteins (LEAs) (Olvera-Carrillo et al. 2011) and heat shock protein (HSPs)/chaperones (Zhu 2016; Jacob et al. 2017). The second group consists of genes that are involved in signaling cascades, e.g., calcium-dependent protein kinase (CDPK) (Ludwig et al. 2004; Asano et al. 2012) and MAPK (Danquah et al. 2014), or TFs, i.e., genes that regulate another gene's transcription (Shinozaki and Yamaguchi-Shinozaki 2000). The third group consists of genes involved cellular homeostasis, e.g., aquaporins and ion transporters (Shah et al. 2017).

3.7.1 *Late-Embryogenesis-Abundant (LEA) Proteins Can Confer Abiotic Stress Tolerance in the Genus Brassica*

LEA proteins are a family of hydrophilic proteins associated with seed desiccation tolerance and play a protective role under salt, cold, and osmotic stresses in the genus *Brassica*. The expression analysis of *LEA4-1*, derived from *B. napus,* revealed that abscisic acid (ABA), salt, cold, and osmotic stresses all induce expression of *LEA4-1* gene in leaf tissues, whereas the reproductive tissues such as flowers and developing seeds showed a constitutive expression of *LEA4* that was upregulated in flowers placed under salt stress (Dalal et al. 2009). Such findings are consistent with studies examining the role of other LEAs in abiotic stress tolerance. Of the nine classified LEA groups, Groups 1, 2, and 3 have been shown to play a role in tolerance to abiotic stresses in other plants. A Group 1 LEA wheat protein PMA1959 was shown to increase the drought and salinity tolerances of transgenic rice (Cheng et al. 2002). Group 2 LEA wheat protein PMA80 conferred drought and salinity tolerances in transgenic rice (Cheng et al. 2002); chimeric double constructs overexpressing either *RAB18* and *COR47* or *LTI29* and *LTI30* conferred freezing stress tolerance to *A. thaliana* (Puhakainen et al. 2004); cold tolerance was conferred to transgenic cucumber seedlings expressing a pGT::*Dhn24* gene fusion (which encodes a SK_3-type DHN24 dehydrin) derived from *Solanum sogarandinum*—a wild potato species native to central to northern Peru (Yin et al. 2006).

3.7.2 *Calcium-Dependent Protein Kinases (CDPKs) Can Confer Abiotic Stress Tolerances in the Genus Brassica*

Second messengers are molecules that relay signals received at receptors located on the cell membrane to target molecules in the cytosol and/or nucleus of the cell.

The arrival of protein hormones, growth factors, or other signals is relayed to the cytosol in what is referred to as a signal cascade. Calcium is a universal second messenger and plays a key role in the signal transduction pathways in plants (Hetherington and Brownlee 2004). Ca^{2+} signaling systems are composed of a receptor located at the cellular membrane that responds to protein, hormone, or other external cellular signals. In turn, a system for propagation of the signal increases the concentration of Ca^{2+} in the cytosol, and downstream mechanisms then react to the increased concentration of Ca^{2+} in the cytosol. Other cellular systems are then responsible for attenuating the signal by returning Ca^{2+} cytosol concentration back to the pre-stimulus level (Sanders et al. 1999). CDPK is one of the four classes of Ca^{2+} receptors or binding proteins known to exist, with the other three classes comprised of calmodulins (CaM), calmodulin-like proteins, and calcineurin B-like proteins (Zielinski 1998; Hrabak et al. 2003; McCormack and Braam 2003; Kolukisaoglu et al. 2004). CDPKs are unique to the other three classes, functioning without requiring an independent calmodulin, because they contain both a protein kinase domain and a calmodulin-like domain in a single polypeptide, acting in both the direct Ca^{2+}-binding and Ca^{2+}-stimulated kinase activities (Roberts and Harmon 1992; Hamel et al. 2014).

In the genus *Brassica*, a genome-wide survey of *B. rapa* var. *rapa* identified 55 *BrCDPK* genes clustered into four subfamilies by phylogenetic analysis. RT-qPCR expression analyses confirmed that all of the identified *BrCDPK* genes responded to several of the tested abiotic stresses (cold, salt, drought, ABA, pst DC3000, 1-aminocyclopropane-1-carboxylic acid (ACC; the precursor of ET), JA, and SA) with transcriptional upregulation (Wu et al. 2017). To examine tolerance to the phytotoxic effects of SO_2 and salt stress, Tseng et al. (2007) introduced the maize *Cu/ZnSOD* and/or *CAT* genes into the chloroplasts of Chinese cabbage cultivar (*B. rapa* var. *pekinensis* cv. Tropical Pride), with the resultant SOD + CAT plants exhibiting an increased tolerance to SO_2 (up to 400 ppb) and visible damage one-sixth that of the WT Chinese cabbage plants. The SOD + CAT plants also showed increased tolerance to salinity after exposure to a high salt treatment of 200 mM NaCl for 4 weeks, with the photosynthetic activity of the SOD + CAT plants decreasing by 6% in comparison to a 72% reduction in WT Chinese cabbage plants (Tseng et al. 2007). Taken together these results suggest that CDPKs are involved in the stress responses of *B. rapa* to various abiotic stressors, with different CDPKs responding to multiple, albeit different abiotic stresses.

3.7.3 Abscisic Acid (ABA) Signaling and ABA-Dependent and Independent Transcription Factors

The ABA pathway is an evolutionarily conserved central regulator of abiotic stress response in plants and acts to mediate many of the responses in abiotic stress signaling (Wasilewska et al. 2008; Danquah et al. 2014). A regulon is a group of genes that

are all regulated by the same regulatory protein. Two such abiotic stress-responsive regulons are controlled by ABA. The first contains the ABA-responsive element-binding proteins (AREB) and the ABA-binding factors (ABF), and the second is composed of the myelocytomatosis oncogene (MYC) and myeloblastosis oncogene (MYB) regulon. TFs are proteins with a DNA domain that binds to a recognition site located in the promoter region of a target gene, acting as either activators or repressors by regulating the transcriptional activity of the target gene, thereby regulating the target gene's expression. The targets of ABA are TFs in the abiotic stress response that activate genes containing ABA-responsive elements (ABRE) or MYC-responsive (MYCR)/MYB-responsive (MYBR) regions within their promoters (Shinozaki and Yamaguchi-Shinozaki 2007; Fujita et al. 2013).

In *B. rapa*, genes involved in ABA signaling have been identified. Using transgenic *A. thaliana* with overexpression of one of the two AtHAB2-like proteins in *B. rapa, BrHAB2a* (Bra025964), was shown to be a putative negative regulator of ABA signaling conferring ABA insensitivity, suggesting that BrHAB2a functions as a protein phosphatase type 2C (PP2C-A), a key component of ABA signaling (Li et al. 2018a). During times of drought, plants reduce water loss via transpiration through the closure of stoma in a process known as stomatal closure. Each stoma is bordered by a pair of guard cells that shrink in response to ABA that is produced in response to drought stress, causing them to become flaccid and the stomatal opening to close. A metabolomic study of drought-stressed *B. napus*, utilizing gas chromatography–mass spectrometry (GC-MS/MS) and LC-MS/MS, identified metabolic signatures in response to ABA in guard cell protoplasts, suggesting that ABA comprises part of the complex signaling pathway of drought response in *B. napus* (Zhu and Assmann 2017). The previously mentioned Group 4 LEA genes studied in *B. napus,* involved in both drought and salt tolerance, are also ABA-induced, further supporting the role of ABA as one of the central signaling pathways in abiotic stress responses (Dalal et al. 2009).

3.7.4 Aquaporins and Ion Transporters, and Their Role in the Abiotic Stress Response of the Genus Brassica

Aquaporins are strongly conserved in both prokaryotes and eukaryotes, and are integral membrane proteins that function as channels in the transfer of water, small solutes, gasses, and ions across the cellular membrane (Takata et al. 2004; Afzal et al. 2016). Aquaporins are part of the highly conserved major intrinsic protein (MIP) superfamily of membrane proteins and are grouped by their localization within the cell. Aquaporins localized to the plasma membrane are further classified into three subgroups, nodulin-26 like intrinsic proteins (NIPs), plasma membrane intrinsic proteins (PIPs), and the uncategorized X intrinsic proteins (XIPs), and are prevalently found on the entirety of the cell surface. Small basic intrinsic proteins (SIPs) are

localized to the endoplasmic reticulum (ER). Aquaporins localized to the membrane of vacuole, i.e., the tonoplast, are tonoplast intrinsic proteins (TIPs).

In plants, aquaporins are involved in the abiotic stress responses of drought, salinity, cold, and osmotic stress, functioning to provide osmotic and nutrient homeostasis. To that end, the transgenic overexpression of various aquaporin genes derived from several plants has generally conferred improved drought tolerance to transfected host plants, e.g., overexpression of a tomato *SlTIP2;2* in transgenic tomato plants (Sade et al. 2009), wheat *TaAQP7* (*PIP2*) overexpressed in tobacco (Zhou et al. 2012), and the overexpression of *Vicia faba PIP1* (*VfPIP1*) in *A. thaliana* by preventing water loss through transpiration due to the induction of stomatal closure (Cui et al. 2008). Similarly, the *B. napus* aquaporin BnPIP1 conferred drought tolerance to transgenic tobacco plants, whereas the *BnPIP1* antisense construct caused developmental abnormalities, altered leaf morphology, and decreased drought tolerance (Yu et al. 2005). Likewise, the overexpression of *Panax ginseng* aquaporin, *PgTIP1*, improved both salt and drought tolerances (Peng et al. 2007) and cold tolerance in transgenic *A. thaliana* plants overexpressing *AtPIP1;4* or *AtPIP2;5* with the latter study noting that, converse to other studies regarding drought tolerance, reduced drought tolerance was observed due to rapid water loss under drought conditions and most likely explained by an increase in hydraulic conductivity (Aharon et al. 2003). Lastly, a tolerance to borate toxicity in *A. thaliana* plants overexpressing *AtTIP5;1* was observed, suggesting that TIPs may be involved in the vacuolar compartmentation of borate (Pang et al. 2010).

A cDNA-AFLP analysis following cadmium (Cd) treatment in *B. juncea* showed the transcription of drought- and ABA-responsive genes in response to exposure to Cd (Fusco et al. 2005). The aquaporins PIP1 and PIP2, denoted as BjCdR51 and BjCdR49 in *B. juncea*, were found to be transcribed in response to Cd stress for a day. This observation coupled with expression of the ABA and drought-responsive genes, aldehyde dehydrogenase BjCdR39 and RNA-binding BjCdR55, suggesting that Cd stress imposes water stress, triggering the ABA stress response pathway.

3.7.5 Heat Stress Response

Heat stress responses in plants have been studied for decades, but most of these studies examine the HSP accumulation, signal transduction, and TFs (Kotak et al. 2007; Nakashima et al. 2014; Dong et al. 2015). HSPs and chaperones are ubiquitous among the prokaryotes and eukaryotes, where they primarily act to ensure proper protein conformation after translation and resolve protein aggregates (Jacob et al. 2017). Based on the number of complete plant genomes and EST sequences currently available, there are 30 known heat stress transcription factors (HSF) encoding genes in Chinese cabbage (Huang et al. 2015).

Using two Chinese cabbage inbred lines, “Chiifu” and “Kenshin”, 51 genes (from 130,000 *Brassica rapa* ESTs) were selected to examine the differences in heat stress responses using RT-PCR. In both lines given heat stress treatment, six, eleven, and

three genes were induced, stimulated, and reduced, respectively (Lee et al. 2010). Using the same Chinese cabbage inbred lines, different thermo-tolerances were profiled by transcriptome analysis to examine the transcriptional changes brought about by heat stress. Leaf disks (1 cm in diameter) incubated at 45 °C for 0.5, 1, 2, 3, or 4 h by floating on a water bath showed enrichment for the GO terms "response to heat," "response to reactive oxygen species (ROS)," "response to temperature stimulus," "response to abiotic stimulus," and "MAPKKK cascade." Most upregulated genes in response to heat stress were HSFs in both lines. Expression of the TF genes Bra024224 (*MYB41*) and Bra021735 (*a bZIP/AIR1* (*Anthocyanin-Impaired-Response-1*)) were specific in the more heat-tolerant Kenshin lines, suggesting that HSFs and specific TF genes may be responsible for conferring heat tolerance in *B. rapa* (Dong et al. 2015). In Indian mustard, several-fold upregulation of the *HSP101* was observed under heat stress (Bhardwaj et al. 2015).

DNA methylation has a significant effect on the genetic expression of plants in response to different abiotic stresses (Dowen et al. 2012; Karan et al. 2012; Shan et al. 2013b; Garg et al. 2015). DNA methylation patterns are altered under heat stress (Gao et al. 2014; Parkin et al. 2014; Li et al. 2016c; Liu et al. 2017a). Liu et al. (2018) analyzed differential methylation and gene expression in non-heading Chinese cabbage under heat stress and revealed the involvement of the different sets of differentially methylated genes at the early and late stages of heat stress. Changes to DNA methylation occurred by heat stress, affecting a large number and diverse set of genes in *B. napus* (Gao et al. 2014).

Tissue-specific changes in the expression of the *B. rapa* noncoding RNA fragments were found, and the most significant changes were observed in $tRNA^{Glu}$ and $tRNA^{Asp}$ under heat stress (Byeon et al. 2018a). Their analysis of tRNA fragments (tRFs) also confirmed that three isoacceptors ($tRF5^{´Asp(GUC)}$, $tRF^{Gly(UCC)}$, and $tRF^{Pseudo(UCC)}$) were severely underrepresented in heat-stressed tissues. The size of the tRF reads was changed significantly in the heat-stressed progeny, while tRFs mapping significantly increased to $tRNA^{Asp}$ and decreased to $tRNA^{Ala}$, $tRNA^{Arg}$, and $tRNA^{Tyr}$ (Byeon et al. 2018b). On the other hand, their enrichment analysis resulted in the significant difference in tRFs processing from $tRNA^{Ala(AGC)}$, $tRNA^{Ala(UGC)}$, $tRNA^{Arg(UGC)}$, $tRNA^{Thr(UGU)}$, $tRNA^{Pseudo(UCC)}$, and $tRNA^{Val(CAC)}$ isoacceptors. The expression of tRFs and snoRNA fragments (snoRFs) is changed by heat stress in *B. rapa* plant progenies but neither of small nuclear RNA fragments (snRFs) and ribosomal RNA fragments (rRFs) (Byeon et al. 2018b). Recently, it has been found that various types of ncRNAs like miRNAs, siRNAs, lncRNAs, and circular RNAs (circRNAs) play a vital role in heat response (de Lima et al. 2012; Khraiwesh et al. 2012). In *B. rapa*, miR398 and its target *CSDs* (i.e., *miR398-CSD/CCS* pathway) were found in the involvement of the heat stress responses, whereas miR156h and miR156g were found to be upregulated and *BracSPL2* were downregulated (Yu et al. 2012). Stief et al. (2014) reported that miR156 can sustainably express the heat stress-responsive genes through *SPL* genes, especially *SPL2* and *SPL11* in *A. thaliana*. Furthermore, 34 specifically expressed lncRNAs and 192 lncRNAs-regulated target genes were identified in *B. rapa* under heat stress (Song et al. 2016b). In cabbage, heat stress-tolerant lines have stronger expression levels for a transcript of *BoHsp70* and TF *BoGRAS*

(*SCL13*) than that of the heat stress-sensitive lines when under heat stress but the expression levels is much lower at young stages (Park et al. 2013). The expression pattern of *BolSGT1* genes in *B. oleracea* was analyzed under heat stress and found that *BolSGT1a* is highly upregulated until 1 h of heat stress treatments, and then subsequently decreased (Shanmugam et al. 2016).

Conversely to the increased heat tolerance conferred by HSFs, a transgenic study overexpressing *SlHsfA3*, derived from *Solanum lycopersicum* (tomato), in *A. thaliana* showed overexpression resulted in an increased heat tolerance and a late flowering phenotype, and sensitivity to salinity in germinating *A. thaliana* plants was increased, suggesting that HSFs are involved in other biological processes related to abiotic stress response (Li et al. 2013c). In *B. napus*, 6-week-old plants were treated with drought via no watering, and leaves were harvested at 3, 5, 7, 10, 12, and 14 days; the subsequent RT-qPCR transcriptional and LC-MS/MS proteomics analyses showed both differentially expressed HSP transcription levels and protein concentrations at the early stages of drought, with decreased transcription during prolonged drought conditions, suggesting that HSPs are initially upregulated in response to drought stress, most likely as a defensive response to maintain cellular homeostasis (Koh et al. 2015). Further supporting the idea that HSPs are involved in several abiotic stress responses, the expression of an HSP gene (*HSP17.4*) was found to be upregulated during drought stress in *B. juncea*, with transcripts present only in the drought-stressed plants. Upon rehydration, transcriptional levels of *HSP17.4* were undetectable. Furthermore, the drought-tolerant variety showed a higher transcript accumulation in comparison to the sensitive variety, with drought-induced changes in gene expression in two contrasting genotypes correlating to the physiological responses of each cultivar (Aneja et al. 2015). *HSP17.4* is a member of the class-I small heat shock protein (sHSP) family and encoded by At3g46230 in *A. thaliana* (Yu et al. 2013). These results in *B. juncea* coupled with similar results in *B. rapa* suggest that it is the small molecular weight HSPs and HSFs that likely comprise the abiotic stress response in the genus *Brassica*, and that the response of these small molecular weight proteins is not limited to only heat stress.

3.8 Perspective

The anticipated climatic changes due to increases in mean global temperature pose a challenge to agricultural production of *Brassica* vegetables. Improved knowledge of the genes and regulatory pathways involved with response to drought, salinity, nutrient deficiency, and temperature (both heat and cold) are fundamental to the success of directed breeding programs aimed at mitigating the impacts of climate change. The efforts that have successfully identified key regulatory genes and beneficial alleles for use in MAS in staple crops such as wheat, rice, and maize may prove useful in *Brassica* vegetables. However, a careful and thorough evaluation of potential markers should be carried out to confirm their usefulness in the context of a *Brassica* vegetable breeding program. In addition, a multidisciplinary approach would

be beneficial, allowing for a more proactive setting of the goals for future breeding programs by utilizing projected climate changes within a given crop's region of cultivation.

References

Abe M, Kobayashi Y, Yamamoto S, Daimon Y, Yamaguchi A, Ikeda Y, Ichinoki H, Notaguchi M, Goto K, Araki T (2005) FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. Science 309:1052–1056

Afrin KS, Rahim MA, Park JI, Natarajan S, Kim HT, Nou IS (2018) Identification of NBS-encoding genes linked to black rot resistance in cabbage (*Brassica oleracea* var. *capitata*). Mol Biol Rep 45:773–785

Afzal Z, Howton T, Sun Y, Mukhtar M (2016) The roles of aquaporins in plant stress responses. J Dev Biol 4:9

Aharon R, Shahak Y, Wininger S, Bendov R, Kapulnik Y, Galili G (2003) Overexpression of a plasma membrane aquaporin in transgenic tobacco improves plant vigor under favorable growth conditions but not under drought or salt stress. Plant Cell 15:439–447

Altpeter F, Springer NM, Bartley LE, Blechl AE, Brutnell TP, Citovsky V, Conrad LJ, Gelvin SB, Jackson DP, Kausch AP, Lemaux PG, Medford JI, Orozco-Cárdenas ML, Tricoli DM, Van Eck J, Voytas DF, Walbot V, Wang K, Zhang ZJ, Stewart CN Jr (2016) Advancing crop transformation in the era of genome editing. Plant Cell 28:1510–1520

Alvarez AM, Benedict AA, Mizumoto CY, Hunter JE, Gabriel DW (1994) Serological, pathological, and genetic diversity among strains of *Xanthomonas campestris* infecting crucifers. Mol Plant Pathol 84:1449–1457

Amoah S, Kurup S, Lopez CM, Welham SJ, Powers SJ, Hopkins CJ, Wilkinson MJ, King GJ (2012) A hypomethylated population of *Brassica rapa* for forward and reverse epi-genetics. BMC Plant Biol 12:193

Andrés F, Coupland G (2012) The genetic basis of flowering responses to seasonal cues. Nat Rev Genet 13:627–639

Aneja B, Yadav NR, Kumar N, Yadav RC (2015) Hsp transcript induction is correlated with physiological changes under drought stress in Indian mustard. Physiol Mol Biol Plants 21:305–316

Asano T, Hayashi N, Kikuchi S, Ohsugi R (2012) CDPK-mediated abiotic stress signaling. Plant Signal Behav 7:817–821

Ayers GW (1957) Races of *Plasmodiophora brassicae*. Can J Bot 35:923–932

Baird NA, Etter PD, Atwood TS, Currey MC, Shiver AL, Lewis ZA, Selker EU, Cresko WA, Johnson EA (2008) Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS ONE 3:e3376

Baranwal VK, Mikkilineni V, Zehr UB, Tyagi AK, Kapoor S (2012) Heterosis: emerging ideas about hybrid vigour. J Exp Bot 63:6309–6314

Barth S, Busimi AK, UtzH Friedrich, Melchinger AE (2003) Heterosis for biomass yield and related traits in five hybrids of *Arabidopsis thaliana* L. Heynh. Heredity 91:36–42

Bateman AJ (1955) Self-incompatibility systems in angiosperms. III. Cruciferae. Heredity 9:52–68

Bäurle I, Dean C (2006) The timing of developmental transitions in plants. Cell 125:655–664

Bayer PE, Golicz AA, Tirnaz S, Chan CK, Edwards D, Batley J (2018) Variation in abundance of predicted resistance genes in the *Brassica oleracea* pangenome. Plant Biotechnol J 17:789–800

Beauchemin C, Boutet N, Laliberté JF (2007) Visualization of the interaction between the precursors of VPg, the viral protein linked to the genome of *turnip mosaic virus*, and the translation eukaryotic initiation factor iso 4E in planta. J Virol 81:775–782

Becker C, Hagmann J, Müller J, Koenig D, Stegle O, Borgwardt K, Weigel D (2011) Spontaneous epigenetic variation in the *Arabidopsis thaliana* methylome. Nature 480:245–249

Bernatavichute YV, Zhang X, Cokus S, Pellegrini M, Jacobsen SE (2008) Genome-wide association of histone H3 lysine nine methylation with CHG DNA methylation in *Arabidopsis thaliana.* PLoS One 3:e3156

Bhardwaj AR, Joshi G, Kukreja B, Malik V, Arora P, Pandey R, Shukla RN, Bankar KG, Katiyar-Agarwal S, Goel S, Jagannath A, Kumar A, Agarwal M (2015) Global insights into high temperature and drought stress regulated genes by RNA-Seq in economically important oilseed crop *Brassica juncea*. BMC Plant Biol 15:9

Blank LM (1937) Fusarium resistance in Wisconsin all seasons cabbage. J Agri Res 55:497–510

Blümel M, Dally N, Jung C (2015) Flowering time regulation in crops-what did we learn from Arabidopsis? Curr Opin Biotechnol 32:121–129

Bosland PW, Williams PH, Morrison RH (1988) Influence of soil temperature on the expression of yellows and wilt of crucifers by *Fusarium oxysporum*. Plant Dis 72:777–780

Bradbury JF (1986) Guide to plant pathogenic bacteria. CAB International Mycological Institute

Buczacki ST, Toxopeus H, Mattusch P, Johnston TD, Dixon GR, Hobolth LA (1975) Study of physiological specialization in *Plasmodiophora brassicae*: proposals for attempted rationalization through an international approach. Trans Br Mycol Soc 65:295–303

Burlakoti RR, Chen JR, Hsu CF, Burlakoti P, Kenyon L (2018) Molecular characterization, comparison of screening methods, and evaluation of cross-pathogenicity of black rot (Xanthomonas campestris pv. campestris) strains from cabbage, choy sum, leafy mustard, and pak choi from Taiwan. Plant Pathol 67:1589–1600

Byeon B, Bilichak A, Kovalchuk I (2018a) Tissue-specific heat-induced changes in the expression of ncRNA fragments in *Brassica rapa* plants. Biocatal Agri Biotechnol 14:338–356

Byeon B, Bilichak A, Kovalchuk I (2018b) Transgenerational response to heat stress in the form of differential expression of noncoding RNA fragments in *Brassica rapa* plants. Plant Genome 12:180022

Camargo LEA, Williams PH, Osborn TC (1995) Mapping of quantitative trait loci controlling resistance of *Brassica oleracea* to *Xanthomonas campestri* pv. *campestris* in the field and green house. Genetics 85:1296–1300

Cao TS, Srivastava S, Rahman MH, Kav NNV, Hotte N, Deyholos MK, Strelkov SE (2008) Proteome-level changes in the roots of *Brassica napus* as a result of *Plasmodiophora brassicae* infection. Plant Sci 174:97–115

Chalhoub B, Denoeud F, Liu S, Parkin IA, Tang H, Wang X, Chiquet J, Belcram H, Tong C, Samans B, Corréa M, Da Silva C, Just J, Falentin C, Koh CS, Le Clainche I, Bernard M, Bento P, Noel B, Labadie K, Alberti A, Charles M, Arnaud D, Guo H, Daviaud C, Alamery S, Jabbari K, Zhao M, Edger PP, Chelaifa H, Tack D, Lassalle G, Mestiri I, Schnel N, Le Paslier MC, Fan G, Renault V, Bayer PE, Golicz AA, Manoli S, Lee TH, Thi VH, Chalabi S, Hu Q, Fan C, Tollenaere R, Lu Y, Battail C, Shen J, Sidebottom CH, Wang X, Canaguier A, Chauveau A, Bérard A, Deniot G, Guan M, Liu Z, Sun F, Lim YP, Lyons E, Town CD, Bancroft I, Wang X, Meng J, Ma J, Pires JC, King GJ, Brunel D, Delourme R, Renard M, Aury JM, Adams KL, Batley J, Snowdon RJ, Tost J, Edwards D, Zhou Y, Hua W, Sharpe AG, Paterson AH, Guan C, Wincker P (2014) Early allopolyploid evolution in the post-Neolithic *Brassica napus* oilseed genome. Science 345:950–953

Charoenporn C, Kanokmedhakul S, Lin FC, Poeaim S, Soytong K (2010) Evaluation of bio-agent formulations to control Fusarium wilt of tomato. Afr J Biotechnol 9:5836–5844

Chen J, Jing J, Zhan Z, Zhang T, Zhang C, Piao Z (2013) Identification of novel QTLs for isolate-specific partial resistance to *Plasmodiophora brassicae* in *Brassica rapa*. PLoS ONE 8:e85307

Chen J, Piao Y, Liu Y, Li X, Piao Z (2018) Genome-wide identification and expression analysis of chitinase gene family in *Brassica rapa* reveals its role in clubroot resistance. Plant Sci 270:257–267

Chen SW, Liu T, Gao Y, Zhang C, Peng SD, Bai MB, Li SJ, Xu L, Zhou XY, Lin LB (2016) Discovery of clubroot-resistant genes in *Brassica napus* by transcriptome sequencing. Genet Mol Res 15:gmr.15038243

Chen X, Ge X, Wang J, Tan C, King GJ, Liu K (2015) Genome-wide DNA methylation profiling by modified reduced representation bisulfite sequencing in *Brassica rapa* suggests that epigenetic modifications play a key role in polyploid genome evolution. Front Plant Sci 6:836

Cheng F, Sun R, Hou X, Zheng H, Zhang F, Zhang Y, Liu B, Liang J, Zhuang M, Liu Y, Liu D, Wang X, Li P, Liu Y, Lin K, Bucher J, Zhang N, Wang Y, Wang H, Deng J, Liao Y, Wei K, Zhang X, Fu L, Hu Y, Liu J, Cai C, Zhang S, Zhang S, Li F, Zhang H, Zhang J, Guo N, Liu Z, Liu J, Sun C, Ma Y, Zhang H, Cui Y, Freeling MR, Borm T, Bonnema G, Wu J, Wang X (2016) Subgenome parallel selection is associated with morphotype diversification and convergent crop domestication in *Brassica rapa* and *Brassica oleracea*. Nat Genet 48:1218–1224

Cheng F, Wu J, Fang L, Sun S, Liu B, Lin K, Bonnema G, Wang X (2012) Biased gene fractionation and dominant gene expression among the subgenomes of *Brassica rapa*. PLoS ONE 7:e36442

Cheng F, Wu J, Wang X (2014) Genome triplication drove the diversification of *Brassica* plants. Hor Res 1:14024

Cheng Y, Xie N, Jin P, Wang T (2015) DNA methylation and hydroxymethylation in stem cells. Cell Biochem Funct 33:161–173

Cheng Z, Targoll J, Huang X, Wu R (2002) Wheat LEA genes, PMA80 and PMA1959, enhance dehydration tolerance of transgenic rice (*Oryza sativa* L.). Mol Breed 10:71–82

Chiang MS, Crête R (1976) Diallel analysis of the inheritance of resistance to race 6 of *Plasmodiophora brassicae* in cabbage. Can J Plant Sci 56:865–868

Chisholm ST, Coaker G, Day B, Staskawicz BJ (2006) Host-microbe interactions: shaping the evolution of the plant immune response. Cell 124:803–814

Chodavarapu RK, Feng S, Ding B, Simon SA, Lopez D, Jia Y, Wang GL, Meyers BC, Jacobsen SE, Pellegrini M (2012) Transcriptome and methylome interactions in rice hybrids. Proc Natl Acad Sci USA 109:12040–12045

Chookajorn T, Kachroo A, Ripoll DR, Clark AG, Nasrallah JB (2004) Specificity determinants and diversification of the *Brassica* self-incompatibility pollen ligand. Proc Natl Acad Sci USA 101:911–917

Chu M, Song T, Falk KC, Zhang X, Liu X, Chang A, Lahlali R, McGregor L, Gossen BD, Yu F, Peng G (2014) Fine mapping of *Rcr1* and analyses of its effect on transcriptome patterns during infection by *Plasmodiophora brassicae*. BMC Genom 15:1166

Chung H, Jeong YM, Mun JH, Lee SS, Chung WH, Yu HJ (2014) Construction of a genetic map based on high-throughput SNP genotyping and genetic mapping of a TuMV resistance locus in *Brassica rapa*. Mol Genet Genom 289:149–160

Cokus SJ, Feng S, Zhang X, Chen Z, Merriman B, Haudenschild CD, Pradhan S, Nelson SF, Pellegrini M, Jacobsen SE (2008) Shotgun bisulphite sequencing of the *Arabidopsis* genome reveals DNA methylation patterning. Nature 452:215–219

Cole SJ, Diener AC (2013) Diversity in receptor-like kinase genes is a major determinant of quantitative resistance to *Fusarium oxysporum* f. sp. *matthioli*. New Phytol 200:172–184

Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, Zhang F (2013) Multiplex genome engineering using CRISPR/Cas systems. Science 339:819–823

Corbesier L, Coupland G (2005) Photoperiodic flowering of *Arabidopsis*: integrating genetic and physiological approaches to characterization of the floral stimulus. Plant Cell Environ 28:54–66

Corbesier L, Vincent C, Jang S, Fornara F, Fan Q, Searle I, Giakountis A, Farrona S, Gissot L, Turnbull C, Coupland G (2007) FT protein movement contributes to long-distance signaling in floral induction of *Arabidopsis*. Science 316:1030–1033

Crow JF (1998) 90 years ago: the beginning of hybrid maize. Genetics 148:923–928

Cruz R, Tenreiro R, Cruz L (2017) Assessment of *Xanthomonas campestris* pathovars affecting cruciferous plants in Portugal and disclosure of two novel *X. campestris* pv. *campestris* races. J Plant Pathol 99:403–414

Cui XH, Hao FS, Chen H, Chen J, Wang XC (2008) Expression of the *Vicia fabaVfPIP1* gene in *Arabidopsis thaliana* plants improves their drought resistance. J Plant Res 121:207–214

Dalal M, Tayal D, Chinnusamy V, Bansal KC (2009) Abiotic stress and ABA-inducible Group 4 *LEA* from *Brassica napus* plays a key role in salt and drought tolerance. J Biotechnol 139:137–145

Daly P, Tomkins B (1995) Production and postharvest handling of Chinese cabbage (*Brassica rapa* var. *pekinensis*). RIRDC 97:41

Danquah A, de Zelicourt A, Colcombet J, Hirt H (2014) The role of ABA and MAPK signaling pathways in plant abiotic stress responses. Biotechnol Adv 32:40–52

Dapp M, Reinders J, Bédiée A, Balsera C, Bucher E, Theiler G, Granier C, Paszkowski J (2015) Heterosis and inbreeding depression of epigenetic *Arabidopsis* hybrids. Nat Plants 1:15092

Das T, Majumdar MH, Devi RT, Rajesh T (2016) Climate change impacts on plant diseases. SAARC J Agri 14:200–209

de Lima JC, Loss-Morais G, Margis R (2012) microRNAs play critical roles during plant development and in response to abiotic stresses. Genet Mol Biol 35(Suppl):1069–1077

De Lucia F, Crevillen P, Jones AM, Greb T, Dean C (2008) A PHD-polycomb repressive complex 2 triggers the epigenetic silencing of *FLC* during vernalization. Proc Natl Acad Sci USA 105:16831–16836

DeLucia EH, Nabity PD, Zavala JA, Berenbaum MR (2012) Climate change: resetting plant-insect interactions. Plant Physiol 160:1677–1685

Deng W, Ying H, Helliwell CA, Taylor JM, Peacock WJ, Dennis ES (2011) FLOWERING LOCUS C (FLC) regulates development pathways throughout the life cycle of *Arabidopsis*. Proc Natl Acad Sci USA 108:6680–6685

Diederichsen E, Frauen M, Linders EGA, Hatakeyama K, Hirai M (2009) Status and perspectives of clubroot resistance breeding in crucifer crops. J Plant Growth Regul 28:265–281

Diener AC (2013) Routine mapping of Fusarium wilt resistance in BC 1 populations of *Arabidopsis thaliana*. BMC Plant Biol 13:171

Diener AC, Ausubel FM (2005) *RESISTANCE TO FUSARIUM OXYSPORUM 1*, a dominant *Arabidopsis* disease-resistance gene, is not race specific. Genetics 171:305–321

Dixon GR (1980) Variation in *Plasmodiophora brassicae*. Ann Appl Biol 94:278–280

Donald EC, Cross SJ, Lawrence JM, Porter IJ (2006) Pathotypes of *Plasmodiophora brassicae*, the cause of clubroot, in Australia. Ann Appl Biol 148:239–244

Dong MA, Farré EM, Thomashow MF (2011) Circadian clock-associated 1 and late elongated hypocotyl regulate expression of the C-repeat binding factor (CBF) pathway in *Arabidopsis*. Proc Natl Acad Sci USA 108:7241–7246

Dong X, Yi H, Lee J, Nou IS, Han CT, Hur Y (2015) Global gene-expression analysis to identify differentially expressed genes critical for the heat stress response in *Brassica rapa*. PLoS ONE 10:e0130451

Doucet R, Shattuck VI, Stobbs LW (1990) Rutabaga germplasm TuMV-R possessing resistance to turnip mosaic virus. Hortic Sci 25:583–584

Doullah MAU, Mohsin GM, Ishikawa K, Hori H, Okazaki K (2011) Construction of a linkage map and QTL analysis for black rot resistance in *Brassica oleracea* L. Intl J Nat Sci 1:1–6

Dowen RH, Pelizzola M, Schmitz RJ, Lister R, Dowen JM, Nery JR, Dixon JE, Ecker JR (2012) Widespread dynamic DNA methylation in response to biotic stress. Proc Natl Acad Sci USA 109:E2183–E2191

Du J, Johnson LM, Jacobsen SE, Patel DJ (2015) DNA methylation pathways and their crosstalk with histone methylation. Nat Rev Mol Cell Biol 16:519–532

Duvick DN (2001) Biotechnology in the 1930s: the development of hybrid maize. Nat Rev Genet 2:69–74

Enya J, Togawa M, Takeuchi T, Yoshida S, Tsushima S, Arie T, Sakai T (2008) Biological and phylogenetic characterization of *Fusarium oxysporum* complex, which causes yellows on *Brassica* spp., and proposal of *F. oxysporum* f. sp. *rapae*, a novel forma specialis pathogenic on *B. rapa* in Japan. Phytopathology 98:475–483

Fargier E, Manceau C (2007) Pathogenicity assays restrict the species *Xanthomonas campestris* into three pathovars and reveal nine races within *X. campestris* pv. *campestris*. Plant Pathol 56:805–818

Farnham MW, Keinath AP, Smith JP (2001) Characterization of Fusarium yellows resistance in collard. Plant Dis 85:890–894

Feng S, Cokus SJ, Zhang X, Chen PY, Bostick M, Goll MG, Hetzel J, Jain J, Strauss SH, Halpern ME, Ukomadu C, Sadler KC, Pradhan S, Pellegrini M, Jacobsen SE (2010) Conservation and divergence of methylation patterning in plants and animals. Proc Natl Acad Sci USA 107:8689–8694

Figdore SS, Ferreira ME, Slocum MK, Williams PH (1993) Association of RFLP markers with trait loci affecting clubroot resistance and morphological characters in *Brassica oleracea* L. Euphytica 69:33–44

Finnegan EJ, Dennis ES (2007) Vernalization-induced trimethylation of histone H3 lysine 27 at *FLC* is not maintained in mitotically quiescent cells. Curr Biol 17:1978–1983

Fjellstrom RG, Williams PH (1997) Fusarium yellows and turnip mosaic virus resistance in *Brassica rapa* and *B. juncea*. HortSci 32:927–930

Flint-Garcia SA, Buckler ES, Tiffin P, Ersoz E, Springer NM (2009) Heterosis is prevalent for multiple traits in diverse maize germplasm. PLoS One 4:e7433

Flor HH (1971) Current status of the gene-for-gene concept. Annu Rev Phytopathol 9:275–296

Fornara F, de Montaigu A, Coupland G (2010) SnapShot: control of flowering in Arabidopsis. Cell 141:550

Franks SJ, Weis AE (2009) Climate change alters reproductive isolation and potential gene flow in an annual plant. Evol Appl 2:481–488

Fravel D, Olivain C, Alabouvette C (2003) *Fusarium oxysporum* and its biocontrol. New Phytol 157:493–502

Friend DJC (1985) Brassica. In: Harlevy AH (ed) Handbook of Flowering. CRC Press, Boca Raton, FL, USA, pp 44–77

Fuchs J, Demidov D, Houben A, Schubert I (2006) Chromosomal histone modification patterns–from conservation to diversity. Trends Plant Sci 11:199–208

Fujimoto R, Kinoshita Y, Kawabe A, Kinoshita T, Takashima K, Nordborg M, Nasrallah ME, Shimizu KK, Kudoh H, Kakutani T (2008a) Evolution and control of imprinted *FWA* genes in the genus *Arabidopsis*. PLoS Genet 4:e1000048

Fujimoto R, Nishio T (2003) Identification of *S* haplotypes in *Brassica* by dot blot analysis of *SP11* alleles. Theor Appl Genet 106:1433–1437

Fujimoto R, Nishio T (2007) Self-Incompatibility. Adv Bot Res 45:139–154

Fujimoto R, Okazaki K, Fukai E, Kusaba M, Nishio T (2006a) Comparison of the genome structure of the self-incompatibility (*S*) locus in interspecific pairs of *S* haplotypes. Genetics 173:1157–1167

Fujimoto R, Sasaki T, Inoue H, Nishio T (2008b) Hypomethylation and transcriptional reactivation of retrotransposon-like sequences in *ddm1* transgenic plants of *Brassica rapa*. Plant Mol Biol 66:463–473

Fujimoto R, Sasaki T, Ishikawa R, Osabe K, Kawanabe T, Dennis ES (2012a) Molecular mechanisms of epigenetic variation in plants. Intl J Mol Sci 13:9900–9922

Fujimoto R, Sasaki T, Kudoh H, Taylor JM, Kakutani T, Dennis ES (2011a) Epigenetic variation in the *FWA* gene within the genus Arabidopsis. Plant J 66:831–843

Fujimoto R, Sugimura T, Fukai E, Nishio T (2006b) Suppression of gene expression of a recessive *SP11/SCR* allele by an untranscribed*SP11/SCR* allele in *Brassica* self-incompatibility. Plant Mol Biol 61:577–587

Fujimoto R, Sugimura T, Nishio T (2006c) Gene conversion from *SLG* to *SRK* resulting in self-compatibility in *Brassica rapa*. FEBS Lett 580:425–430

Fujimoto R, Takuno S, Sasaki T, Nishio T (2008c) The pattern of amplification and differentiation of *Ty1-copia* and *Ty3-gypsy* retrotransposons in Brassicaceae species. Genes Genet Syst 83:13–22

Fujimoto R, Taylor JM, Sasaki T, Kawanabe T, Dennis ES (2011b) Genome wide gene expression in artificially synthesized amphidiploids of *Arabidopsis*. Plant Mol Biol 77:419–431

Fujimoto R, Taylor JM, Shirasawa S, Peacock WJ, Dennis ES (2012b) Heterosis of *Arabidopsis* hybrids between C24 and Col is associated with increased photosynthesis capacity. Proc Natl Acad Sci USA 109:7109–7114

Fujimoto R, Uezono K, Ishikura S, Osabe K, Peacock WJ, Dennis ES (2018) Recent research on the mechanism of heterosis is important for crop and vegetable breeding systems. Breed Sci 68:145–158

Fujita Y, Yoshida T, Yamaguchi-Shinozaki K (2013) Pivotal role of the AREB/ABF-SnRK2 pathway in ABRE-mediated transcription in response to osmotic stress in plants. Physiol Plant 147:15–27

Fujiwara A, Inukai T, Kim BM, Masuta C (2011) Combinations of a host resistance gene and the CI gene of turnip mosaic virus differentially regulate symptom expression in *Brassica rapa* cultivars. Arch Virol 156:1575–1581

Fujiwara S, Oda A, Yoshida R, Niinuma K, Miyata K, Tomozoe Y, Tajima T, Nakagawa M, Hayashi K, Coupland G, Mizoguchi T (2008) Circadian clock proteins LHY and CCA1 regulate SVP protein accumulation to control flowering in *Arabidopsis*. Plant Cell 20:2960–2971

Fukai E, Fujimoto R, Nishio T (2003) Genomic organization of the *S* core region and the *S* flanking regions of a class-II *S* haplotype in *Brassica rapa*. Mol Genet Genom 269:361–369

Fusco N, Micheletto L, Dal Corso G, Borgato L, Furini A (2005) Identification of cadmium-regulated genes by cDNA-AFLP in the heavy metal accumulator *Brassica juncea* L. J Exp Bot 56:3017–3027

Gao G, Li J, Li H, Li F, Xu K, Yan G, Chen B, Qiao J, Wu X (2014) Comparison of the heat stress induced variations in DNA methylation between heat-tolerant and heat-sensitive rapeseed seedlings. Breed Sci 64:125–133

Garg R, Chevala VN, Shankar R, Jain M (2015) Divergent DNA methylation patterns associated with gene expression in rice cultivars with contrasting drought and salinity stress response. Sci Rep 5:14922

Gendall AR, Levy YY, Wilson A, Dean C (2001) The *VERNALIZATION 2* gene mediates the epigenetic regulation of vernalization in *Arabidopsis*. Cell 107:525–535

Giraud H, Bauland C, Falque M, Madur D, Combes V, Jamin P, Monteil C, Laborde J, Palaffre C, Gaillard A, Blanchard P, Charcosset A, Moreau L (2017) Reciprocal genetics: Identifying QTL for general and specific combining abilities in hybrids between multiparental populations from two maize (*Zea mays* L.) heterotic groups. Genetics 207:1167–1180

Girke A, Schierholt A, Becker HC (2012) Extending the rapeseed gene pool with resynthesized *Brassica napus* II: heterosis. Theor Appl Genet 124:1017–1026

Glazebrook J (2005) Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu Rev Phytopathol 43:205–227

Golicz AA, Bayer PE, Barker GC, Edger PP, Kim H, Martinez PA, Chan CK, Severn-Ellis A, McCombie WR, Parkin IA, Paterson AH, Pires JC, Sharpe AG, Tang H, Teakle GR, Town CD, Batley J, Edwards D (2016) Thepangenome of an agronomically important crop plant *Brassica oleracea*. Nat Commun 7:13390

Grandclément C, Thomas G (1996) Detection and analysis of QTLs based on RAPD markers for polygenic resistance to *Plasmodiophora brassicae* Woron in *Brassica oleracea* L. Theor Appl Genet 93:86–90

Greaves IK, Groszmann M, Ying H, Taylor JM, Peacock WJ, Dennis ES (2012) Trans chromosomal methylation in *Arabidopsis* hybrids. Proc Natl Acad Sci USA 109:3570–3575

Green RM, Tobin EM (2002) The role of CCA1 and LHY in the plant circadian clock. Dev Cell 2:516–518

Green SK, Deng TC (1985) Turnip mosaic virus strains in cruciferous hosts in Taiwan. Plant Dis 69:28–31

Griffiths PD, Marek LF, Robertson LD (2009) Identification of crucifer accessions from the NC-7 and NE-9 plant introduction collections that are resistant to black rot (*Xanthomonas campestris* pv. *campestris*) races 1 and 4. Hortic Sci 44:284–288

Groszmann M, Gonzalez-Bayon R, Greaves IK, Wang L, Huen AK, Peacock WJ, Dennis ES (2014) Intraspecific Arabidopsis hybrids show different patterns of heterosis despite the close relatedness of the parental genomes. Plant Physiol 166:265–280

Groszmann M, Greaves IK, Albert N, Fujimoto R, Helliwell CA, Dennis ES, Peacock WJ (2011) Epigenetics in plants-vernalisation and hybrid vigour. Biochim Biophys Acta 1809:427–437

Groszmann M, Greaves IK, Fujimoto R, Peacock WJ, Dennis ES (2013) The role of epigenetics in hybrid vigour. Trends Genet 29:684–690

Grover JW, Kendall T, Baten A, Burgess D, Freeling M, King GJ, Mosher RA (2018) Maternal components of RNA directed DNA methylation are required for seed development in *Brassica rapa*. Plant J 94:575–582

Guo H, Dickson MH, Hunter JE (1991) *Brassica napus* sources of resistance to black rot in crucifers and inheritance of resistance. Hortic Sci 26:1545–1547

Guo Y, Chen S, Li Z, Cowling WA (2014) Center of origin and centers of diversity in an ancient crop, *Brassica rapa* (Turnip Rape). J Hered 105:555–565

Hamel LP, Sheen J, Séguin A (2014) Ancient signals: comparative genomics of green plant CDPKs. Trends Plant Sci 19:79–89

Hamlyn BMG (1953) Quantitative studies on the transmission of cabbage black ring spot virus by *Myzus persicae* (Sulz.). Ann Appl Biol 40:393–402

Hansen M (1989) Genetic variation and inheritance of tolerance to clubroot (*Plasmodiophora brassicae* Wor.) and other quantitative character in cabbage(*Brassica oleracea* L). Hereditas 110:13–22

Harris RA, Wang T, Coarfa C, Nagarajan RP, Hong C, Downey SL, Johnson BE, Fouse SD, Delaney A, Zhao Y, Olshen A, Ballinger T, Zhou X, Forsberg KJ, Gu J, Echipare L, O'Geen H, Lister R, Pelizzola M, Xi Y, Epstein CB, Bernstein BE, Hawkins RD, Ren B, Chung WY, Gu H, Bock C, Gnirke A, Zhang MQ, Haussler D, Ecker JR, Li W, Farnham PJ, Waterland RA, Meissner A, Marra MA, Hirst M, Milosavljevic A, Costello JF (2010) Comparison of sequencing-based methods to profile DNA methylation and identification of monoallelic epigenetic modifications. Nat Biotechnol 28:1097–1105

Haseyama Y, Kitashiba H, Okamoto S, Tonouchi E, Sakamoto K, Nishio T (2018) Nucleotide sequence analysis of *S*-locus genes to unify *S* haplotype nomenclature in radish. Mol Breed 38:116

Hatakeyama K, Horisaki A, Niikura S, Narusaka Y, Abe H, Yoshiaki H, Ishida M, Fukuoka H, Matsumoto S (2010) Mapping of quantitative trait loci for high level of self-incompatibility in *Brassica rapa* L. Genome 53:257–265

Hatakeyama K, Niwa T, Kato T, Ohara T, Kakizaki T, Matsumoto S (2017) The tandem repeated organization of NB-LRR genes in the clubroot-resistant *CRb* locus in *Brassica rapa* L. Mol Geneti Genom 292:397–405

Hatakeyama K, Suwabe K, Tomita RN, Kato T, Nunome T, Fukuoka H, Matsumoto S (2013) Identification and characterization of *CRR1a*, a gene for resistance to clubroot disease (*Plasmodiophora brassicae* Woronin) in *Brassica rapa* L. PLoS ONE 8:e54745

Hatakeyama K, Takasaki T, Suzuki G, Nishio T, Watanabe M, Isogai A, Hinata K (2001) The *S* receptor kinase gene determines dominance relationships in stigma expression of self-incompatibility in *Brassica*. Plant J 26:69–76

Hatakeyama K, Takasaki T, Watanabe M, Hinata K (1998) Molecular characterization of *S* locus genes, *SLG* and *SRK*, in a pollen-recessive self-incompatibility haplotype of *Brassicarapa* L. Genetics 149:1587–1597

He G, Elling AA, Deng XW (2011) The epigenome and plant development. Annu Rev Plant Biol 62:411–435

He G, Zhu X, Elling AA, Chen L, Wang X, Guo L, Liang M, He H, Zhang H, Chen F, Qi Y, Chen R, Deng XW (2010) Global epigenetic and transcriptional trends among two rice subspecies and their reciprocal hybrids. Plant Cell 22:17–33

Helliwell CA, Wood CC, Robertson M, James Peacock W, Dennis ES (2006) The Arabidopsis FLC protein interacts directly in vivo with SOC1 and FT chromatin and is part of a high-molecular-weight protein complex. Plant J 46:183–192

Heo JB, Sung S (2011) Vernalization-mediated epigenetic silencing by a long intronic noncoding RNA. Science 331:76–79

Hepworth SR, Valverde F, Ravenscroft D, Mouradov A, Coupland G (2002) Antagonistic regulation of flowering-time gene SOC1 by CONSTANS and FLC via separate promoter motifs. EMBO J 21:4327–4337

Hetherington AM, Brownlee C (2004) The generation of Ca^{2+} signals in plants. Annu Rev Plant Biol 55:401–427

Hinata K, Okazaki K, Nishio T (1983) Gene analysis of self-compatibility in *Brassicacampestris* var. *yellow sarson* (a case of recessive epistatic modifier). In: Proceedings of the 6th International Rapeseed Conference, vol 1, pp 354–359

Hirai M, Harada T, Kubo N, Tsukada M, Suwabe K, Matsumoto S (2004) A novel locus for clubroot resistance in *Brassica rapa* and its linkage markers. Theor Appl Genet 108:639–643

Howden SM, Soussana JF, Tubiello FN, Chhetri N, Dunlop M, Meinke H (2007) Adapting agriculture to climate change. Proc Natl Acad Sci USA 104:19691–19696

Hrabak EM, Chan CW, Gribskov M, Harper JF, Choi JH, Halford N, Kudla J, Luan S, Nimmo HG, Sussman MR, Thomas M, Walker-Simmons K, Zhu JK, Harmon AC (2003) The Arabidopsis CDPK-SnRK superfamily of protein kinases. Plant Physiol 132:666–680

Huang XY, Tao P, Li BY, Wang WH, Yue ZC, Lei JL, Zhong XM (2015) Genome-wide identification, classification, and analysis of heat shock transcription factor family in Chinese cabbage (*Brassica rapa pekinensis*). Genet Mol Res 14:2189–2204

Huang Z, Peng G, Liu X, Deora A, Falk KC, Gossen BD, McDonald MR, Yu F (2017) Fine mapping of a clubroot resistance gene in Chinese cabbage using SNP markers identified from bulked segregant RNA sequencing. Front Plant Sci 8:1448

Hughes SL, Hunter PJ, Sharpe AG, Kearsey MJ, Lydiate DJ, Walsh JA (2003) Genetic mapping of the novel turnip mosaic virus resistance gene *TuRB03* in *Brassica napus*. Theor Appl Genet 107:1169–1173

Huijser P, Schmid M (2011) The control of developmental phase transitions in plants. Development 138:4117–4129

Hunter JE, Dickson MH, Ludwig JW (1987) Source of resistance to black rot of cabbage expressed in seedlings and adult plants. Plant Dis 71:263–266

Ignatov A, Kuginuki Y, Hida K (1998) Race-specific reaction of resistance to black rot in *Brassica oleracea*. Eur J Plant Pathol 104:821–827

Imaizumi T (2010) Arabidopsis circadian clock and photoperiodism: time to think about location. Curr Opi Plant Biol 13:83–89

Irwin JA, Soumpourou E, Lister C, Ligthart JD, Kennedy S, Dean C (2016) Nucleotide polymorphism affecting *FLC* expression underpins heading date variation in horticultural brassicas. Plant J 87:597–605

Itabashi E, Osabe K, Fujimoto R, Kakizaki T (2018) Epigenetic regulation of agronomical traits in Brassicaceae. Plant Cell Rep 37:87–101

Jacob P, Hirt H, Bendahmane A (2017) The heat-shock protein/chaperone network and multiple stress resistance. Plant Biotechnol J 15:405–414

Jamwal RS, Sharma PP (1986) Inheritance of resistance to black rot (*Xanthomonas campestris* pv. *Campestris*) in cauliflower (*Brassica oleracea* var. *Botrytis*). Euphytica 35:941–943

Jan FJ, Fagoaga C, Pang SZ, Gonsalves D (2000) A single chimeric transgene derived from two distinct viruses confers multi-virus resistance in transgenic plants through homology-dependent gene silencing. J Gen Virol 81:2103–2109

Jeddeloh JA, Stokes TL, Richards EJ (1999) Maintenance of genomic methylation requires a SWI2/SNF2-like protein. Nat Genet 22:94–97

Jenner CE, Sánchez F, Nettleship SB, Foster GD, Ponz F, Walsh JA (2000) The cylindrical inclusion gene of *Turnip mosaic virus* encodes a pathogenic determinant to the Brassica resistance gene *TuRB01*. Mol Plant Microbe Interact 13:1102–1108

Jenner CE, Walsh JA (1996) Pathotypic variation in turnip mosaic virus with special reference to European isolates. Plant Pathol 45:848–856

Jenner CE, Wang X, Tomimura K, Ohshima K, Ponz F, Walsh JA (2003) The dual role of the potyvirus P3 protein of *Turnip mosaic virus* as a symptom and avirulence determinant in Brassicas. Mol Plant Microbe Interact 16:777–784

Jenner CE, Wang XW, Ponz F, Walsh JA (2002) A fitness cost for *Turnip mosaic virus* to overcome host resistance. Virus Res 86:1–6

Jensen BD, Vicente JG, Manandhar HK, Roberts SJ (2010) Occurrence and diversity of *Xanthomonas campestris* pv. *campestris* in vegetable *Brassica* fields in Nepal. Plant Dis 94:298–305

Jia H, Wei X, Yang Y, Yuan Y, Wei F, Zhao Y, Yang S, Yao Q, Wang Z, Tian B, Zhang X (2017) Root RNA-seq analysis reveals a distinct transcriptome landscape between clubroot-susceptible and clubroot-resistant Chinese cabbage lines after *Plasmodiophora brassicae* infection. Plant Soil 421:93–105

Jiang H, Song W, Li A, Yang X, Sun D (2011) Identification of genes differentially expressed in cauliflower associated with resistance to *Xanthomonas campestris* pv. *campestris*. Mol Biol Rep 38:621–629

Jin M, Lee SS, Ke L, Kim JS, Seo MS, Sohn SH, Park BS, Bonnema G (2014) Identification and mapping of a novel dominant resistance gene, *TuRB07* to Turnip mosaic virus in *Brassica rapa*. Theor Appl Genet 127:509–519

Johannes F, Porcher E, Teixeira FK, Saliba-Colombani V, Simon M, Agier N, Bulski A, Albuisson J, Heredia F, Audigier P, Bouchez D, Dillmann C, Guerche P, Hospital F, Colot V (2009) Assessing the impact of transgenerational epigenetic variation on complex traits. PLoS Genet 5:e1000530

Johnson R (1984) A critical analysis of durable resistance. Ann Rev Phytopathol 22:309–330

Jones DF (1917) Dominance of linked factors as a means of accounting for heterosis. Genetics 2:466–479

Jones JDG, Dangl JL (2006) The plant immune system. Nature 444:323–329

Jung C, Müller AE (2009) Flowering time control and applications in plant breeding. Trends Plant Sci 14:563–573

Kachroo A, Schopfer CR, Nasrallah ME, Nasrallah JB (2001) Allele-specific receptor-ligand interaction in *Brassica* self-incompatibility. Science 293:1824–1826

Kageyama K, Asano T (2009) Life cycle of *Plasmodiophora brassicae*. J Plant Growth Regul 28:203–211

Kakita M, Murase K, Iwano M, Matsumoto T, Watanabe M, Shiba H, Isogai A, Takayama S (2007a) Two distinct forms of *M*-locus protein kinase localize to the plasma membrane and interact directly with *S*-locus receptor kinase to transduce self-incompatibility signaling in *Brassica rapa*. Plant Cell 19:3961–3973

Kakita M, Shimosato H, Murase K, Isogai A, Takayama S (2007b) Direct interaction between *S*-locus receptor kinase and *M*-locus protein kinase involved in *Brassica* self-incompatibility signaling. Plant Biotechnol 24:185–190

Kakizaki T, Kato T, Fukino N, Ishida M, Hatakeyama K, Matsumoto S (2011) Identification of quantitative trait loci controlling late bolting in Chinese cabbage (*Brassica rapa* L.) parental line Nou 6 gou. Breed Sci 61:151–159

Kakizaki T, Takada Y, Ito A, Suzuki G, Shiba H, Takayama S, Isogai A, Watanabe M (2003) Linear dominance relationship among four class-II *S* haplotypes in pollen is determined by the expression of *SP11* in *Brassica* self-incompatibility. Plant Cell Physiol 44:70–75

Kalia P, Saha P, Ray S (2017) Development of RAPD and ISSR derived SCAR markers linked to Xca1Bo gene conferring resistance to black rot disease in cauliflower (*Brassica oleracea* var. *botrytis* L.). Euphytica 213:232

Karan R, Deleon T, Biradar H, Subudhi PK (2012) Salt stress induced variation in DNA methylation pattern and its influence on gene expression in contrasting rice genotypes. PLoS ONE 7:e40203

Kardailsky I, Shukla VK, Ahn JH, Dagenais N, Christensen SK, Nguyen JT, Chory J, Harrison MJ, Weigel D (1999) Activation tagging of the floral inducer *FT*. Science 286:1962–1965

Kato T, Hatakeyama K, Fukino N, Matsumoto S (2012) Identification of a clubroot resistance locus conferring resistance to a *Plasmodiophora brassicae* classified into pathotype group 3 in Chinese cabbage (*Brassica rapa* L.). Breed Sci 62:282–287

Kato T, Hatakeyama K, Fukino N, Matsumoto S (2013) Fine mapping of the clubroot resistance gene *CRb* and development of a useful selectable marker in *Brassica rapa*. Breed Sci 63:116–124

Kawamura K, Kawanabe T, Shimizu M, Nagano AJ, Saeki N, Okazaki K, Kaji M, Dennis ES, Osabe K, Fujimoto R (2016) Genetic distance of inbred lines of Chinese cabbage and its relationship to heterosis. Plant Gene 5:1–7

Kawamura K, Kawanabe T, Shimizu M, Okazaki K, Kaji M, Dennis ES, Osabe K, Fujimoto R (2015) Genetic characterization of inbred lines of Chinese cabbage by DNA markers; towards the application of DNA markers to breeding of F_1 hybrid cultivars. Data Brief 6:229–237

Kawamura K, Shimizu M, Kawanabe T, Pu Z, Kodama T, Kaji M, Osabe K, Fujimoto R, Okazaki K (2017) Assessment of DNA markers for seed contamination testing and selection of disease resistance in cabbage. Euphytica 213:228

Kawanabe T, Ishikura S, Miyaji N, Sasaki T, Wu LM, Itabashi E, Takada S, Shimizu M, Takasaki-Yasuda T, Osabe K, Peacock WJ, Dennis ES, Fujimoto R (2016a) Role of DNA methylation in hybrid vigor in *Arabidopsis thaliana*. Proc Natl Acad Sci USA 113:E6704–E6711

Kawanabe T, Osabe K, Itabashi E, Okazaki K, Dennis ES, Fujimoto R (2016b) Development of primer sets that can verify the enrichment of histone modifications, and their application to examining vernalization-mediated chromatin changes in *Brassica rapa* L. Genes Genet Syst 91:1–10

Kemp DB, Eichenseer K, Kiessling W (2015) Maximum rates of climate change are systematically underestimated in the geological record. Nat Commun 6:8890

Khraiwesh B, Zhu JK, Zhu J (2012) Role of miRNAs and siRNAs in biotic and abiotic stress responses of plants. Biochim Biophys Acta 1819:137–148

Kifuji Y, Hanzawa H, Terasawa Y, Ashutosh Nishio T (2013) QTL analysis of black rot resistance in cabbage using newly developed EST-SNP markers. Euphytica 190:289–295

Kim DH, Doyle MR, Sung S, Amasino RM (2009) Vernalization: winter and the timing of flowering in plants. Annu Rev Cell Dev 25:277–299

Kim DH, Sung S (2017) Vernalization-triggered intragenic chromatin loop formation by long noncoding RNAs. Dev Cell 40:302–312

Kim J, Kang WH, Hwang J, Yang HB, Dosun K, Oh CS, Kang BC (2014) Transgenic Brassica rapa plants over-expressing eIF(iso)4E variants show broad-spectrum Turnip mosaic virus (TuMV) resistance. Mol Plant Pathol 15:615–626

Kim J, Kang WH, Yang HB, Park S, Jang CS, Yu HJ, Kang BC (2013) Identification of a broad-spectrum recessive gene in *Brassica rapa* and molecular analysis of the eIF4E gene family to develop molecular markers. Mol Breed 32:385–398

Kimura R, Sato K, Fujimoto R, Nishio T (2002) Recognition specificity of self-incompatibility maintained after the divergence of *Brassicaoleracea* and *Brassicarapa*. Plant J 29:215–223

Kitamoto N, Nishikawa K, Tanimura Y, Urushibara S, Matsuura T, Yokoi S, Takahata Y, Yui S (2017) Development of late-bolting F1 hybrids of Chinese cabbage(*Brassica rapa* L.) allowing early spring cultivation without heating. Euphytica 213:292

Kitamoto N, Yui S, Nishikawa K, Takahata Y, Yokoi S (2014) A naturally occurring long insertion in the first intron in the *Brassica rapaFLC2* gene causes delayed bolting. Euphytica 196:213–223

Kobayashi Y, Kaya H, Goto K, Iwabuchi M, Araki T (1999) A pair of related genes with antagonistic roles in mediating flowering signals. Science 286:1960–1962

Koh J, Chen G, Yoo MJ, Zhu N, Dufresne D, Erickson JE, Shao H, Chen S (2015) Comparative proteomic analysis of *Brassica napus* in response to drought stress. J Proteome Res 14:3068–3081

Kole C, Quijada P, Michaels SD, Amasino RM, Osborn TC (2001) Evidence for homology of flowering-time genes *VFR2* from *Brassica rapa* and *FLC* from *Arabidopsis thaliana*. Theor Appl Genet 102:425–430

Kolukisaoglu U, Weinl S, Blazevic D, Batistic O, Kudla J (2004) Calcium sensors and their interacting protein kinases: genomics of the Arabidopsis and rice CBL-CIPK signaling networks. Plant Physiol 134:43–58

Kotak S, Larkindale J, Lee U, von Koskull-Döring P, Vierling E, Scharf KD (2007) Complexity of the heat stress response in plants. Curr Opin Plant Biol 10:310–316

Kuginuki Y, Ajisaka H, Yui M, Yoshikawa H, Hida K, Hirai M (1997) RAPD markers linked to a clubroot-resistance locus in *Brassica rapa* L. Euphytica 98:149–154

Kuginuki Y, Yoshikawa H, Hirai M (1999) Variation in virulence of *Plasmodiophora brassicae* in Japan tested with clubroot-resistant cultivars of Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*). Eur J Plant Pathol 105:327–332

Kusaba M, Nishio T (1999) Comparative analysis of *S* haplotypes with very similar *SLG* alleles in *Brassicarapa* and *Brassicaoleracea*. Plant J 17:83–91

Kusaba M, Nishio T, Satta Y, Hinata K, Ockendon DJ (1997) Striking sequence similarity in inter- and intra-specific comparisons of class I *SLG* alleles from *Brassica oleracea* and *Brassica campestris:* Implication for the evolution and recognition mechanism. Proc Natl Acad Sci USA 94:7673–7678

Lai AG, Doherty CJ, Mueller-Roeber B, Kay SA, Schippers JH, Dijkwel PP (2012) *CIRCADIAN CLOCK-ASSOCIATED 1* regulates ROS homeostasis and oxidative stress responses. Proc Natl Acad Sci USA 109:17129–17134

Laird PW (2010) Principles and challenges of genome-wide DNA methylation analysis. Nat Rev Genet 11:191–203

Lao X, Suwabe K, Niikura S, Kakita M, Iwano M, Takayama S (2014) Physiological and genetic analysis of CO_2-induced breakdown of self-incompatibility in *Brassica rapa*. J Exp Bot 65:939–951

Laurens F, Thomas G (1993) Inheritance of resistance to clubroot (*Plasmodiophora brassicae* Wor.) in kale (*Brassica oleracea* ssp. *Acephala*). Hereditas 119:253–262

Lauss K, Wardenaar R, Oka R, van Hulten MHA, Guryev V, Keurentjes JJB, Stam M, Johannes F (2018) Parental DNA methylation states are associated with heterosis in epigenetic hybrids. Plant Physiol 176:1627–1645

Law JA, Jacobsen SE (2010) Establishing, maintaining and modifying DNA methylation patterns in plants and animals. Nat Rev Genet 11:204–220

Lawrenson T, Shorinola O, Stacey N, Li C, Østergaard L, Patron N, Uauy C, Harwood W (2015) Induction of targeted, heritable mutations in barley and *Brassica oleracea* using RNA-guided Cas9 nuclease. Genome Biol 16:258

Lee H, Suh SS, Park E, Cho E, Ahn JH, Kim SG, Lee JS, Kwon YM, Lee I (2000) The AGAMOUS-LIKE 20 MADS domain protein integrates floral inductive pathways in *Arabidopsis*. Genes Dev 14:2366–2376

Lee J, Izzah NK, Choi BS, Joh HJ, Lee SC, Perumal S, Seo J, Ahn K, Jo EJ, Choi GJ, Nou IS, Yu Y, Yang TJ (2016) Genotyping-by-sequencing map permits identification of clubroot resistance QTLs and revision of the reference genome assembly in cabbage (*Brassica oleracea* L.). DNA Res 23:29–41

Lee J, Izzah NK, Jayakodi M, Perumal S, Joh HJ, Lee HJ, Lee SC, Park JY, Yang KW, Nou IS, Seo J, Yoo J, Suh Y, Ahn K, Lee JH, Choi GJ, Yu Y, Kim H, Yang TJ (2015) Genome-wide SNP identification and QTL mapping for black rot resistance in cabbage. BMC Plant Biol 15:32

Lee J, Song H, Han CT, Lim YP, Chung SM, Hur Y (2010) Expression characteristics of heat shock protein genes in two comparable inbred lines of Chinese cabbage, Chiifu and Kenshin. Genes Genom 32:247–257

Lehmann P, Jenner CE, Kozubek E, Greenland AJ, Walsh JA (2003) Coat protein-mediated resistance to *Turnip mosaic virus* in oilseed rape (*Brassica napus*). Mol Breed 11:83–94

Levy YY, Mesnage S, Mylne JS, Gendall AR, Dean C (2002) Multiple roles of *Arabidopsis VRN1* in vernalization and flowering time control. Science 297:243–246

Li E, Ling J, Wang G, Xiao J, Yang Y, Mao Z, Wang X, Xie B (2015a) Comparative proteomics analyses of two races of *Fusarium oxysporum* f. sp. *conglutinans* that differ in pathogenicity. Sci Rep 5:13663

Li E, Wang G, Xiao J, Ling J, Yang Y, Xie B (2016a) A SIX1 homolog in *Fusarium oxysporum* f. sp. *conglutinans* is required for full virulence on cabbage. PLoS One 11:e0152273

Li E, Wang G, Yang Y, Xiao J, Mao Z, Xie B (2015b) Microscopic analysis of the compatible and incompatible interactions between *Fusarium oxysporum* f. sp. *conglutinans* and cabbage. Eur J Plant Pathol 141:597–609

Li F, Kitashiba H, Inaba K, Nishio T (2009) A *Brassica rapa* linkage map of EST-based SNP markers for identification of candidate genes controlling flowering time and leaf morphological traits. DNA Res 6:311–323

Li G, Hu X, Hou L, Cao L, Wang Q, Wang D, Mu X, Zhang Y, Zhou X, Zhao Y, Xie CG (2018a) Molecular identification of BrHAB2a, one of the two AtHAB2-like proteins *in Brassica rapa*, is an important component of ABA signaling. Biochem Biophys Res Commun 503:495–500

Li G, Qian W, Zhang SJ, Zhang SF, Li F, Zhang H, Wu J, Wang XW, Sun RF (2016b) Development of gene-based markers for the Turnip mosaic virus resistance gene retr02 in Brassica rapa. Plant Breed 135:466–470

Li G, Siddiqui H, Teng Y, Lin R, Wan XY, Li J, Lau OS, Ouyang X, Dai M, Wan J, Devlin PF, Deng XW, Wang H (2011) Coordinated transcriptional regulation underlying the circadian clock in *Arabidopsis*. Nat Cell Biol 13:616–622

Li H, Li X, Xuan Y, Jiang J, Wei Y, Piao Z (2018b) Genome wide identification and expression profiling of *SWEET* genes family reveals its role during *Plasmodiophora brassicae*-induced formation of clubroot in *Brassica rapa*. Front Plant Sci 9:207

Li H, Yuan J, Wu M, Han Z, Li L, Jiang H, Jia Y, Han X, Liu M, Sun D, Chen C, Song W, Wang C (2018c) Transcriptome and DNA methylome reveal insights into yield heterosis in the curds of broccoli (*Brassica oleracea* L var. *italic*). BMC Plant Biol 18:168

Li J, Huang Q, Sun M, Zhang T, Li H, Chen B, Xu K, Gao G, Li F, Yan G, Qiao J, Cai Y, Wu X (2016c) Global DNA methylation variations after short-term heat shock treatment in cultured microspores of *Brassica napus* cv. Topas. Sci Rep 6:38401

Li JF, Norville JE, Aach J, McCormack M, Zhang D, Bush J, Church GM, Sheen J (2013a) Multiplex and homologous recombination-mediated genome editing in *Arabidopsis* and *Nicotiana benthamiana* using guide RNA and Cas9. Nat Biotechnol 31:688–691

Li L, Luo Y, Chen B, Xu K, Zhang F, Li H, Huang Q, Xiao X, Zhang T, Hu J, Li F, Wu X (2016d) A genome-wide association study reveals new loci for resistance to clubroot disease in *Brassica napus*. Front Plant Sci 7:1483

Li Q, Zhang X, Zeng Q, Zhang Z, Liu S, Pei Y, Wang S, Liu X, Xu W, Fu W, Zhao Z, Song X (2015c) Identification and mapping of a novel Turnip mosaic virus resistance gene *TuRBCS01* in Chinese cabbage (*Brassica rapa* L.). Plant Breed 134:221–225

Li X, Ramchiary N, Dhandapani V, Choi SR, Hur Y, Nou IS, Yoon MK, Lim YP (2013b) Quantitative trait loci mapping in *Brassica rapa* revealed the structural and functional conservation of genetic loci governing morphological and yield component traits in the A, B, and C subgenomes of *Brassica* species. DNA Res 20:1–16

Li X, Zhang S, Bai J, He Y (2016e) Tuning growth cycles of *Brassica* crops via natural antisense transcripts of *BrFLC*. Plant Biotechnol J 14:905–914

Li Z, Zhang L, Wang A, Xu X, Li J (2013c) Ectopic overexpression of SlHsfA3, a heat stress transcription factor from tomato, confers increased thermotolerance and salt hypersensitivity in germination in transgenic Arabidopsis. PLoS ONE 8:e54880

Lin SI, Wang JG, Poon SY, Su CL, Wang SS, Chiou TJ (2005) Differential regulation of *FLOWERING LOCUS C* expression by vernalization in cabbage and Arabidopsis. Plant Physiol 137:1037–1048

Lindhout P (2002) The perspectives of polygenic resistance in breeding for durable disease resistance. Euphytica 124:217–226

Lippman ZB, Zamir D (2007) Heterosis: revisiting the magic. Trends Genet 23:60–66

Lister R, O'Malley RC, Tonti-Filippini J, Gregory BD, Berry CC, Millar AH, Ecker JR (2008) Highly integrated single-base resolution maps of the epigenome in *Arabidopsis*. Cell 133:523–536

Liu G, Xia Y, Liu T, Dai S, Hou X (2018) The DNA methylome and association of differentially methylated regions with differential gene expression during heat stress in *Brassica rapa*. Intl J Mol Sci 19:1414

Liu J, Kim BM, Kaneko Y, Inukai T, Masuta C (2015) Identification of the *TuNI* gene causing systemic necrosis in *Arabidopsis* ecotype Ler infected with *Turnip mosaic virus* and characterization of its expression. J Gen Plant Pathol 81:180–191

Liu J, Liu X, Dai L, Wang G (2007) Recent progress in elucidating the structure, function and evolution of disease resistance genes in plants. J Genet Genom 34:765–776

Liu S, Liu Y, Yang X, Tong C, Edwards D, Parkin IAP, Zhao M, Ma J, Yu J, Huang S, Wang X, Wang J, Lu K, Fang Z, Bancroft I, Yang TJ, Hu Q, Wang X, Yue Z, Li H, Yang L, Wu J, Zhou Q, Wang W, King GJ, Pires JC, Lu C, Wu Z, Sampath P, Wang Z, Guo H, Pan S, Yang L, Min J, Zhang D, Jin D, Li W, Belcram H, Tu J, Guan M, Qi C, Du D, Li J, Jiang L, Batley J, Sharpe AG, Park BS, Ruperao P, Cheng F, Waminal NE, Huang Y, Dong C, Wang L, Li J, Hu Z, Zhuang M, Huang Y, Huang J, Shi J, Mei D, Liu J, Lee TH, Wang J, Jin H, Li Z, Li X, Zhang J, Xiao L, Zhou Y, Liu Z, Liu X, Qin R, Tang X, Liu W, Wang Y, Zhang Y, Lee J, Kim HH, Denoeud F, Xu X, Liang X, Hua W, Wang X, Wang J, Chalhoub B, Paterson AH (2014) The *Brassica oleracea* genome reveals the asymmetrical evolution of polyploid genomes. Nat Commun 5:3930

Liu T, Li Y, Duan W, Huang F, Hou X (2017a) Cold acclimation alters DNA methylation patterns and confers tolerance to heat and increases growth rate in *Brassica rapa*. J Exp Bot 68:1213–1224

Liu X, Han F, Kong C, Fang Z, Yang L, Zhang Y, Zhuang M, Liu Y, Li Z, Lv H (2017b) Rapid introgression of the Fusarium wilt resistance gene into an elite cabbage line through the combined application of a microspore culture, genome background analysis, and disease resistance-specific marker assisted foreground selection. Front Plant Sci 8:354

Lou P, Wu J, Cheng F, Cressman LG, Wang X, McClung CR (2012) Preferential retention of circadian clock genes during diploidization following whole genome triplication *in Brassica rapa*. Plant Cell 24:2415–2426

Lou P, Zhao J, Kim JS, Shen S, Del Carpio DP, Song X, Jin M, Vreugdenhil D, Wang X, Koornneef M, Bonnema G (2007) Quantitative trait loci for flowering time and morphological traits in multiple populations of *Brassica rapa*. J Exp Bot 58:4005–4016

Lovelock DA, Donald CE, Conlan XA, Cahill DM (2013) Salicylic acid suppression of clubroot in broccoli (*Brassicae oleracea* var. *italica*) caused by the obligate biotroph *Plasmodiophora brassicae*. Austral Plant Pathol 42:141–153

Lowe AJ, Moule C, Trick M, Edwards KJ (2004) Efficient large-scale development of microsatellites for marker and mapping applications in *Brassica* crop species. Theor Appl Genet 108:1103–1112

Lu SX, Webb CJ, Knowles SM, Kim SH, Wang Z, Tobin EM (2012) CCA1 and ELF3 Interact in the control of hypocotyl length and flowering time in Arabidopsis. Plant Physiol 158:1079–1088

Ludwig AA, Romeis T, Jones JDG (2004) CDPK-mediated signalling pathways: specificity and cross-talk. J Exp Bot 55:181–188

Ludwig-Müller J, Bennett RN, Kiddle G, Ihmig S, Ruppel M, Hilgenberg W (1999) The host range of *Plasmodiophora brassicae* and its relationship to endogenous glucosinolate content. New Phytol 141:443–458

Luo Y, Dong D, Su Y, Wang X, Peng Y, Peng J, Zhou C (2018) Transcriptome analysis of *Brassica juncea* var. *tumida* Tsen responses to *Plasmodiophora brassicae* primed by the biocontrol strain *Zhihengliuella aestuarii*. Funct Integr Genom 18:301–314

Lv H, Fang Z, Yang L, Zhang Y, Wang Q, Liu Y, Zhuang M, Yang Y, Xie B, Liu B, Liu J, Kang J, Wang X (2014a) Mapping and analysis of a novel candidate Fusarium wilt resistance gene *FOC1* in *Brassica oleracea*. BMC Genom 15:1094

Lv HH, Wang QB, Yang LM, Fang ZY, Liu YM, Zhuang M, Zhang YY, Yang YH, Xie BY, Wang XW (2014b) Breeding of cabbage (*Brassica oleracea* L. var. *capitata*) with fusarium wilt resistance based on microspore culture and marker-assisted selection. Euphytica 200:465–473

Lv HH, Yang LM, Kang JG, Wang QB, Wang XW, Fang ZY, Liu YM, Zhuang M, Zhang YY, Lin Y, Yang YH, Xie BY, Liu B, Liu JS (2013) Development of InDel markers linked to Fusarium wilt resistance in cabbage. Mol Breed 32:961–967

Lydiate DJ, Pilcher RLR, Higgins EE, Walsh JA (2014) Genetic control of immunity to *Turnip mosaic virus* (TuMV) pathotype 1 in *Brassica rapa* (Chinese cabbage). Genome 57:419–425

Lyons R, Stiller J, Powell J, Rusu A, Manners JM, Kazan K (2015) *Fusarium oxysporum* triggers tissue-specific transcriptional reprogramming in *Arabidopsis thaliana*. PLoS ONE 10:e0121902

Ma J, Hou X, Xiao D, Qi L, Wang F, Sun F, Wang Q (2010) Cloning and characterization of the *BcTuR3* gene related to resistance to Turnip mosaic virus (TuMV) from non-heading Chinese cabbage. Plant Mol Biol Rep 28:588–596

Makarevitch I, Eichten SR, Briskine R, Waters AJ, Danilevskaya ON, Meeley RB, Myers CL, Vaughn MW, Springer NM (2013) Genomic distribution of maize facultative heterochromatin marked by trimethylation of H3K27. Plant Cell 25:780–793

Manoharan RK, Shanmugam A, Hwang I, Park JI, Nou IS (2016) Expression of salicylic acid-related genes in *Brassica oleracea* var. *capitata* during *Plasmodiophora brassicae* infection. Genome 59:379–391

Manzanares-Dauleux MJ, Delourme R, Baron F, Thomas G (2000) Mapping of one major gene and of QTLs involved in resistance to clubroot in *Brassica napus*. Theor Appl Genet 101:885–891

Martín ÁM, y Poch HLC, Herrera DM, Ponz F (1999) Resistances to Turnip mosaic potyvirus in *Arabidopsis thaliana*. Mol Plant Microbe Interact 12:1016–1021

Más P, Devlin PF, Panda S, Kay SA (2000) Functional interaction of phytochrome B and cryptochrome 2. Nature 408:207–211

Massomo SMS, Mortensen NC, Mabagala RB, Newman MA, Hockenhull J (2004) Biological control of black rot (*Xanthomonas campestris* pv. *campestris*) of cabbage in Tanzania with *Bacillus* strains. J Plant Pathol 152:98–105

Matsumoto E, Yasui C, Ohi M, Tsukada M (1998) Linkage analysis of RFLP markers for clubroot resistance and pigmentation in Chinese cabbage (*Brassica rapa* ssp. *pekinensis*). Euphytica 104:79–86

Matzke MA, Mosher RA (2014) RNA-directed DNA methylation: an epigenetic pathway of increasing complexity. Nat Rev Genet 15:394–408

McClung CR (2014) Wheels within wheels: new transcriptional feedback loops in the Arabidopsis circadian clock. F1000prime Rep 6:2

McCormack E, Braam J (2003) Calmodulins and related potential calcium sensors of Arabidopsis. New Phytol 159:585–598

Metzker ML (2010) Sequencing technologies-the next generation. Nat Rev Genet 11:31–46

Meyer RC, Witucka-Wall H, Becher M, Blacha A, Boudichevskaia A, Dörmann P, Fiehn O, Friedel S, von Korff M, Lisec J, Melzer M, Repsilber D, Schmidt R, Scholz M, Selbig J, Willmitzer L, Altmann T (2012) Heterosis manifestation during early Arabidopsis seedling development is characterized by intermediate gene expression and enhanced metabolic activity in the hybrids. Plant J 71:669–683

Michaels SD, Amasino RM (1999) *FLOWERING LOCUS C* encodes a novel MADS domain protein that acts as a repressor of flowering. Plant Cell 11:949–956

Miller M, Song Q, Shi X, Juenger TE, Chen ZJ (2015) Natural variation in timing of stress-responsive gene expression predicts heterosis in intraspecific hybrids of *Arabidopsis*. Nat Commun 6:7453

Miura A, Yonebayashi S, Watanabe K, Toyama T, Shimada H, Kakutani T (2001) Mobilization of transposons by a mutation abolishing full DNA methylation in *Arabidopsis*. Nature 411:212–214

Miyaji N, Fujimoto R (2018) Hybrid vigor: importance of epigenetic processes and consequences for breeding. Adv Bot Res 88:247–275

Miyaji N, Shimizu M, Miyazaki J, Osabe K, Sato M, Ebe Y, Takada S, Kaji M, Dennis ES, Fujimoto R, Okazaki K (2017) Comparison of transcriptome profiles by *Fusarium oxysporum* inoculation between Fusarium yellows resistant and susceptible lines in *Brassica rapa* L. Plant Cell Rep 36:1841–1854

Moll RH, Lonnquist JH, VélezFortuno J, Johnson EC (1965) The relationship of heterosis and genetic divergence in maize. Genetics 52:139–144

Montoya JM, Raffaelli D (2010) Climate change, biotic interactions and ecosystem services. Phil Trans Roy Soc B 365:2013–2018

Moriguchi K, Kimizuka-Takagi C, Ishii K, Nomura K (1999) A genetic map based on RAPD, RFLP, isozyme, morphological markers and QTL analysis for clubroot resistance in *Brassica oleracea*. Breed Sci 49:257–265

Morrison RH, Mengistu A, Williams PH (1994) First report of race 2 of cabbage yellows caused by *Fusarium oxysporum* f. sp. *conglutinans* in Texas. Plant Dis 78:641

Mortazavi A, Williams BA, McCue K, Schaeffer L, Wold B (2008) Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nat Methods 5:621–628

Murase K, Shiba H, Iwano M, Che FS, Watanabe M, Isogai A, Takayama S (2004) A membrane-anchored protein kinase involved in *Brassica* self-incompatibility signaling. Science 303:1516–1519

Mylne JS, Barrett L, Tessadori F, Mesnage S, Johnson L, Bernatavichute YV, Jacobsen SE, Fransz P, Dean C (2006) LHP1, the *Arabidopsis* homologue of HETEROCHROMATIN PROTEIN1, is required for epigenetic silencing of *FLC*. Proc Natl Acad Sci USA 103:5012–5017

Nagaoka T, Doullah MAU, Matsumoto S, Kawasaki S, Ishikawa T, Hori H, Okazaki K (2010) Identification of QTLs that control clubroot resistance in *Brassica oleracea* and comparative analysis of clubroot resistance genes between *B. rapa* and *B. oleracea*. Theor Appl Genet 120:1335–1346

Nakanishi T, Esashi Y, Hinata K (1969) Control of self-incompatibility by CO2 gas in *Brassica*. Plant Cell Physiol 10:925–927

Nakanishi T, Hinata K (1973) An effective time for CO_2 gas treatment in overcoming self-incompatibility in *Brassica*. Plant Cell Physiol 14:873–879

Nakashima K, Yamaguchi-Shinozaki K, Shinozaki K (2014) The transcriptional regulatory network in the drought response and its crosstalk in abiotic stress responses including drought, cold, and heat. Front Plant Sci 5:170

Namazkar S, Egsgaard H, Frenck G, Terkelsen T, Jørgensen RB (2015) Significant reductions in oil quality and lipid content of oilseed rape (*Brassica napus*L.) under climate change. Procedia Environ Sci 29:121–122

Nasrallah JB, Nishio T, Nasrallah ME (1991) The self-incompatibility genes of Brassica: expression and use in genetic ablation of floral tissues. Annu Rev Plant Physiol Plant Mol Biol 42:393–422

Nasrallah JB, Rundle SJ, Nasrallah ME (1994) Genetic evidence for the requirement of the *BrassicaS*-locus receptor kinase gene in the self-incompatibility response. Plant J 5:373–384

Neik TX, Barbetti MJ, Batley J (2017) Current status and challenges in identifying disease resistance genes in *Brassica napus*. Front Plant Sci 8:1788

Nekrasov V, Staskawicz B, Weigel D, Jones JDG, Kamoun S (2013) Targeted mutagenesis in the model plant *Nicotiana benthamiana* using Cas9 RNA-guided endonuclease. Nat Biotechnol 31:691–693

Newbery F, Qi A, Fitt BDL (2016) Modelling impacts of climate change on arable crop diseases: progress, challenges and applications. Curr Opin Plant Biol 32:101–109

Nguyen HD, Tran HTN, Ohshima K (2013) Genetic variation of the *Turnip mosaicvirus* population of Vietnam: a case study of founder, regional and local influences. Virus Res 171:138–149

Nguyen ML, Monakhos GF, Komakhin RA, Monakhos SG (2018) The new clubroot resistance locus is located on chromosome A05 in Chinese cabbage (*Brassica rapa* L.). Russ J Genet 54:296–304

Ni Z, Kim ED, Ha M, Lackey E, Liu J, Zhang Y, Sun Q, Chen ZJ (2009) Altered circadian rhythms regulate growth vigour in hybrids and allopolyploids. Nature 457:327–331

Niederhuth CE, Bewick AJ, Ji L, Alabady MS, Do Kim K, Li Q, Rohr NA, Rambani A, Burke JM, Udall JA, Egesi C, Schmutz J, Grimwood J, Jackson SA, Springer NM, Schmitz RJ (2016) Widespread natural variation of DNA methylation within angiosperms. Genome Biol 17:194

Niikura S, Matsuura S (2000) Genetic analysis of the reaction level of self-incompatibility to a 4% CO_2 gas treatment in the radish (*Raphanus sativus* L.). Theor Appl Genet 101:1189–1193

Nishio T, Kusaba M, Sakamoto K, Ockendon DJ (1997) Polymorphism of the kinase domain of the *S*-locus receptor kinase gene (*SRK*) in *Brassica oleracea* L. Theor Appl Genet 95:335–342

Nishio T, Kusaba M, Watanabe M, Hinata K (1996) Registration of *S* alleles in *Brassica campestris* L by the restriction fragment sizes of *SLGs.*Theor ApplGenet 92:388–394

Niwa Y, Ito S, Nakamichi N, Mizoguchi T, Niinuma K, Yamashino T, Mizuno T (2007) Genetic linkages of the circadian clock-associated genes, *TOC1, CCA1* and *LHY*, in the photoperiodic control of flowering time in *Arabidopsis thaliana*. Plant Cell Physiol 48:925–937

Nomura K, Minegishi Y, Kimizuka-Takagi C, Fujioka T, Moriguchi K, Shishido R, Ikehashi H (2005) Evaluation of F_2 and F_3 plants introgressed with QTLs for clubroot resistance in cabbage developed by using SCAR markers. Plant Breed 124:371–375

Nou IS, Watanabe M, Isogai A, Hinata K (1993) Comparison of *S*-alleles and *S*-glycoproteins between two wild populations of *Brassica campestris* in Turkey and Japan. Sex Plant Reprod 6:79–86

Nyalugwe EP, Barbetti MJ, Jones RAC (2014) Preliminary studies on resistance phenotypes to *Turnip mosaic virus* in *Brassica napus* and *B. carinata* from different continents and effects of temperature on their expression. Eur J Plant Pathol 139:687–706

Nyalugwe EP, Barbetti MJ, Jones RAC (2015a) Studies on resistance phenotypes to *Turnip mosaic virus* in five species of *Brassicaceae*, and identification of a virus resistance gene in *Brassica juncea*. Eur J Plant Pathol 141:647–666

Nyalugwe EP, Barbetti MJ, Jones RAC (2016) Strain specificity of *Turnip mosaic virus* resistance gene *TuRBJU 01* in *Brassica juncea*. Eur J Plant Pathol 145:209–213

Nyalugwe EP, Jones RAC, Barbetti MJ, Kehoe MA (2015b) Biological and molecular variation amongst Australian *Turnip mosaic virus* isolates. Plant Pathol 64:1215–1223

Ockendon DJ (2000) The *S*-allele collection of *Brassica oleracea*. Acta Hortic 539:25–30

Oh S, Park S, van Nocker S (2008) Genic and global functions for Paf1C in chromatin modification and gene expression in Arabidopsis. PLoS Genet 4:e1000077

Ohshima K, Tomitaka Y, Wood JT, Minematsu Y, Kajiyama H, Tomimura K, Gibbs AJ (2007) Patterns of recombination in turnip mosaic virus genomic sequences indicate hotspots of recombination. J Gen Virol 88:298–315

Ohshima K, Yamaguchi Y, Hirota R, Hamamoto T, Tomimura K, Tan Z, Sano T, Azuhata F, Walsh JA, Fletcher J, Chen J, Gera A, Gibbs A (2002) Molecular evolution of *Turnip mosaic virus*: evidence of host adaptation, genetic recombination and geographical spread. J Gen Virol 83:1511–1521

Oikawa E, Takuno S, Izumita A, Sakamoto K, Hanzawa H, Kitashiba H, Nishio T (2011) Simple and efficient methods for *S* genotyping and *S* screening in genus *Brassica* by dot-blot analysis. Mol Breed 28:1–12

Okazaki K, Kusaba M, Ockendon DJ, Nishio T (1999) Characterization of *S* tester line in *Brassica oleracea*: polymorphism of restriction fragment length of *SLG* homologues and isoelectric points of *S*-locus glycoproteins. Theor Appl Genet 98:1329–1334

Okazaki K, Sakamoto K, Kikuchi R, Saito A, Togashi E, Kuginuki Y, Matsumoto S, Hirai M (2007) Mapping and characterization of *FLC* homologs and QTL analysis of flowering time in *Brassica oleracea*. Theor Appl Genet 114:595–608

Okuzaki A, Ogawa T, Koizuka C, Kaneko K, Inaba M, Imamura J, Koizuka N (2018) CRISPER/Cas9-mediated genome editing of the fatty acid desaturase 2 gene in *Brassica napus*. Plant Physiol Biochem 131:63–69

Olvera-Carrillo Y, Reyes JL, Covarrubias AA (2011) Late embryogenesis abundant proteins: versatile players in the plant adaptation to water limiting environments. Plant Signal Behav 6:586–589

Osabe K, Sasaki T, Ishikawa R, Fujimoto R (2012) The role of DNA methylation in plants. In: Tatarinova TV, Sablok G (eds) DNA methylation: principles, mechanisms and challenges. NOVA Publishers, NY, USA, pp 35–66

Osborn TC, Kole C, Parkin IA, Sharpe AG, Kuiper M, Lydiate DJ, Trick M (1997) Comparison of flowering time genes in *Brassica rapa, B. napus* and *Arabidopsis thaliana*. Genetics 146:1123–1129

Oumouloud A, El-Otmani M, Chikh-Rouhou H, Garces Claver A, Gonzalez Torres R, Rerl-Treves R, Alvarez JM (2013) Breeding melon for resistance to Fusarium wilt: recent developments. Euphytica 192:155–169

Pang W, Fu P, Li X, Zhan Z, Yu S, Piao Z (2018) Identification and mapping of the clubroot resistance gene *CRd* in Chinese cabbage (*Brassica rapa* ssp. *pekinensis*). Front Plant Sci 9:653

Pang Y, Li L, Ren F, Lu P, Wei P, Cai J, Xin L, Zhang J, Chen J, Wang X (2010) Overexpression of the tonoplast aquaporin AtTIP5;1 conferred tolerance to boron toxicity in Arabidopsis. J Genet Genom 37:389–397

Paritosh K, Yadava SK, Gupta V, Panjabi-Massand P, Sodhi YS, Pradhan AK, Pental D (2013) RNA-seq based SNPs in some agronomically important oleiferous lines of *Brassica rapa* and their use for genome-wide linkage mapping and specific-region fine mapping. BMC Genom 14:463

Park HJ, Jung WY, Lee SS, Song JH, Kwon SY, Kim H, Kim C, Ahn JC, Cho HS (2013) Use of heat stress responsive gene expression levels for early selection of heat tolerant cabbage (*Brassica oleracea* L.). Int J Mol Sci 14:11871–11894

Park JI, Nou IS, Lee SS, Kang KK, Watanabe M (2001) Identification of *S*-genotypes by PCR-RFLP in breeding lines of Brassica. Mol Cells 12:227–232

Parkin IAP, Koh C, Tang H, Robinson SJ, Kagale S, Clarke WE, Town CD, Nixon J, Krishnakumar V, Bidwell SL, Denoeud F, Belcram H, Links MG, Just J, Clarke C, Bender T, Huebert T, Mason AS, Pires CJ, Barker G, Moore J, Walley PG, Manoli S, Batley J, Edwards D, Nelson MN, Wang X, Paterson AH, King G, Bancroft I, Chalhoub B, Sharpe AG (2014) Transcriptome and methylome profiling reveals relics of genome dominance in the mesopolyploid *Brassica oleracea*. Genome Biol 15:R77

Peng L, Zhou L, Li Q, Wei D, Ren X, Song H, Mei J, Si J, Qian W (2018) Identification of quantitative trait loci for clubroot resistance in *Brassica oleracea* with the use of *Brassica* SNP microarray. Front Plant Sci 9:822

Peng Y, Lin W, Cai W, Arora R (2007) Overexpression of a *Panax ginseng* tonoplast aquaporin alters salt tolerance, drought tolerance and cold acclimation ability in transgenic *Arabidopsis* plants. Planta 226:729–740

Piao Y, Jin K, He Y, Liu J, Liu S, Li X, Piao Z (2018) Genome-wide identification and role of *MKK* and *MPK* gene families in clubroot resistance of *Brassica rapa*. PLoS ONE 13:e0191015

Piao Z, Ramchiary N, Lim YP (2009) Genetics of clubroot resistance in *Brassica* species. J Plant Growth Regul 28:252–264

Piao ZY, Deng YQ, Choi SR, Park YJ, Lim YP (2004) SCAR and CAPS mapping of *CRb*, a gene conferring resistance to *Plasmodiophora brassicae* in Chinese cabbage (*Brassica rapa* ssp. *pekinensis*). Theor Appl Genet 108:1458–1465

Pink DAC, Sutherland RA, Walkey DGA (1986) Genetic analysis of resistance in Brussels sprout to cauliflower mosaic and turnip mosaic viruses. Ann Appl Biol 109:199–208

Pink DAC, Walkey DGA (1990) Resistance to turnip mosaic virus in white cabbage. Euphytica 51:101–107

Pino Del Carpio D, Basnet RK, De Vos RC, Maliepaard C, Visser R, Bonnema G (2011) The patterns of population differentiation in a *Brassica rapa* core collection. Theor Appl Genet 122:1105–1118

Ploetz RC (2006) Fusarium wilt of banana is caused by several pathogens referred to as *Fusarium oxysporum* f. sp. *cubense*. Phytopatholojy 96:653–656

Provvidenti R (1980) Evaluation of Chinese cabbage cultivars from Japan and the People's Republic of China for resistance to turnip mosaic virus and cauliflower mosaic virus. J Amer Soc Hortic Sci 105:571–573

Pu Z, Ino Y, Kimura Y, Tago A, Shimizu M, Natsume S, Sano Y, Fujimoto R, Kaneko K, Shea DJ, Fukai E, Fuji S-I, Hirano H, Okazaki K (2016) Changes in the proteome of xylem sap in *Brassica oleracea* in response to *Fusarium oxysporum* stress. Front Plant Sci 7:31

Pu ZJ, Shimizu M, Zhang YJ, Nagaoka T, Hayashi T, Hori H, Matsumoto S, Fujimoto R, Okazaki K (2012) Genetic mapping of a fusarium wilt resistance gene in *Brassica oleracea*. Mol Breed 30:809–818

Puhakainen T, Hess MW, Mäkelä P, Svensson J, Heino P, Palva ET (2004) Overexpression of multiple dehydrin genes enhances tolerance to freezing stress in Arabidopsis. Plant Mol Biol 54:743–753

Putterill J, Laurie R, Macknight R (2004) It's time to flower: the genetic control of flowering time. BioEssays 26:363–373

Qian W, Zhang S, Zhang S, Li F, Zhang H, Wu J, Wang X, Walsh JA, Sun R (2013) Mapping and candidate-gene screening of the novel Turnip mosaic virus resistance gene *retr02* in Chinese cabbage (*Brassica rapa* L.). Theor Appl Genet 126:179–188

Quadrana L, Colot V (2016) Plant transgenerational epigenetics. Annu Rev Genet 50:467–491

Rafalski A (2002) Applications of single nucleotide polymorphisms in crop genetics. Curr Opin Plant Biol 5:94–100

Rahman M, McVetty PB, Li G (2007) Development of SRAP, SNP and multiplexed SCAR molecular markers for the major seed coat color gene in *Brassica rapa* L. Theor Appl Genet 115:1101–1107

Raman H, Raman R, Coombes N, Song J, Prangnell R, Bandaranayake C, Tahira R, Sundaramoorthi V, Killian A, Meng J, Dennis ES (2016) Genome-wide association analyses reveal complex genetic architecture underlying natural variation for flowering time in canola. Plant Cell Environ 39:1228–1239

Ramchiary N, Nguyen VD, Li X, Hong CP, Dhandapani V, Choi SR, Yu G, Piao ZY, Lim YP (2011) Genic microsatellite markers in *Brassica rapa*: development, characterization, mapping, and their utility in other cultivated and wild *Brassica* relatives. DNA Res 18:305–320

Razi H, Howell EC, Newbury HJ, Kearsey MJ (2008) Does sequence polymorphism of FLC paralogues underlie flowering time QTL in *Brassica oleracea*? Theor Appl Genet 116:179–192

Reinders J, Wulff BBH, Mirouze M, Marí-Ordóñez A, Dapp M, Rozhon W, Bucher E, Theiler G, Paszkowski J (2009) Compromised stability of DNA methylation and transposon immobilization in mosaic *Arabidopsis* epigenomes. Genes Dev 23:939–950

Robaglia C, Caranta C (2006) Translation initiation factors: a weak link in plant RNA virus infection. Trends Plant Sci 11:40–45

Roberts DM, Harmon AC (1992) Calcium-modulated proteins: Targets of intracellular calcium signals in higher plants. Annu Rev Plant Physiol Plant Mol Biol 43:375–414

Rocherieux J, Glory P, Giboulot A, Boury S, Barbeyron G, Thomas G, Manzanares-Dauleux MJ (2004) Isolate-specific and broad-spectrum QTLs are involved in the control of clubroot in *Brassica oleracea*. Theor Appl Genet 108:1555–1563

Rodríguez-Hernández AM, Gosalvez B, Sempere RN, Burgos L, Aranda MA, Truniger V (2012) Melon RNA interference (RNAi) lines silenced for *Cm-eIF4E* show broad virus resistance. Mol Plant Pathol 13:755–763

Rohde P, Hincha DK, Heyer AG (2004) Heterosis in the freezing tolerance of crosses between two *Arabidopsis thaliana* accessions (Columbia-0 and C24) that show differences in non-acclimated and acclimated freezing tolerance. Plant J 38:790–799

Roudier F, Ahmed I, Bérard C, Sarazin A, Mary-Huard T, Cortijo S, Bouyer D, Caillieux E, Duvernois-Berthet E, Al-Shikhley L, Giraut L, Després B, Drevensek S, Barneche F, Dèrozier S, Brunaud V, Aubourg S, Schnittger A, Bowler C, Martin-Magniette ML, Robin S, Caboche M, Colot V (2011) Integrative epigenomic mapping defines four main chromatin states in Arabidopsis. EMBO J 30:1928–1938

Rouhrazi K, Khodakaramian G (2014) Genetic fingerprinting of Iranian *Xanthomonas campestris* pv. *campestris* strains inducing black rot disease of crucifers. Eur J Plant Pathol 139:175–184

Ruffio-Châble V, Hervé Y, Dumas C, Gaude T (1997) Distribution of *S*-haplotypes and its relationship with self-incompatibility in *Brassica oleracea*. Part 1. In inbred lines of cauliflower (*B. oleracea* var 'botrytis'). Theor Appl Genet 94:338–346

Rusholme RL, Higgins EE, Walsh JA, Lydiate DJ (2007) Genetic control of broad-spectrum resistance to turnip mosaic virus in *Brassica rapa* (Chinese cabbage). J Gen Virol 88:3177–3186

Sade N, Vinocur BJ, Diber A, Shatil A, Ronen G, Nissan H, Wallach R, Karchi H, Moshelion M (2009) Improving plant stress tolerance and yield production: is the tonoplast aquaporin SlTIP2; 2 a key to isohydric to anisohydric conversion? New Phytol 181:651–661

Saeki N, Kawanabe T, Ying H, Shimizu M, Kojima M, Abe H, Okazaki K, Kaji M, Taylor JM, Sakakibara H, Peacock WJ, Dennis ES, Fujimoto R (2016) Molecular and cellular characteristics of hybrid vigour in a commercial hybrid of Chinese cabbage. BMC Plant Biol 16:45

Saha P, Kalia P, Sharma M, Singh D (2016) New source of black rot disease resistance in *Brassica oleracea* and genetic analysis of resistance. Euphytica 207:35–48

Saha P, Kalia P, Sonah H, Sharma TR (2014) Molecular mapping of black rot resistance locus *Xca1bo* on chromosome 3 in Indian cauliflower (*Brassica oleracea* var. *botrytis* L.). Plant Breed 133:268–274

Saito M, Kubo N, Matsumoto S, Suwabe K, Tsukada M, Hirai M (2006) Fine mapping of the clubroot resistance gene, *Crr3*, in *Brassica rapa*. Theor Appl Genet 114:81–91

Sakamoto K, Kusaba M, Nishio T (2000) Single-seed PCR-RFLP analysis for the identification of S haplotypes in commercial F_1 hybrid cultivars of broccoli and cabbage. Plant Cell Rep 19:400–406

Sakamoto K, Nishio T (2001) Distribution of S haplotypes in commercial cultivars of *Brassica rapa*. Plant Breed 120:155–161

Sakamoto K, Saito A, Hayashida N, Taguchi G, Matsumoto E (2008) Mapping of isolate-specific QTLs for clubroot resistance in Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*). Theor Appl Genet 117:759–767

Samach A, Onouchi H, Gold SE, Ditta GS, Schwarz-Sommer Z, Yanofsky MF, Coupland G (2000) Distinct roles of CONSTANS target genes in reproductive development of *Arabidopsis*. Science 288:1613–1616

Sanders D, Brownlee C, Harper JF (1999) Communicating with calcium. Plant Cell 11:691–706

Sasaki T, Fujimoto R, Kishitani S, Nishio T (2011) Analysis of target sequences of DDM1s in *Brassica rapa* by MSAP. Plant Cell Rep 30:81–88

Sato K, Nishio T, Kimura R, Kusaba M, Suzuki T, Hatakeyama K, Ockendon DJ, Satta Y (2002) Co-evolution of the *S*-locus genes, *SRK*, *SLG* and *SP11/SCR*, in *Brassicaoleracea* and *B. rapa*. Genetics 162:931–940

Sato Y, Fujimoto R, Toriyama K, Nishio T (2003) Commonality of self-recognition specificity of *S* haplotypes between *Brassica oleracea* and *Brassica rapa*. Plant Mol Biol 52:617–626

Sato Y, Okamoto S, Nishio T (2004) Diversification and alteration of recognition specificity of the pollen ligand SP11/SCR in self-incompatibility of Brassica and Raphanus. Plant Cell 16:3230–3241

Sato Y, Sato K, Nishio T (2006) Interspecific pairs of class II *S* haplotypes having different recognition specificities between *Brassica oleracea* and *Brassica rapa*. Plant Cell Physiol 47:340–345

Schaad NW (1982) Detection of seedborne bacterial plant pathogens. Plant Dis 66:885–890

Scheben A, Wolter F, Batley J, Puchta H, Edwards D (2017) Towards CRISPER/Cas crops-bringing together genomics and genome editing. New Phytol 216:682–698

Schield DR, Walsh MR, Card DC, Andrew AL, Adams RH, Castoe TA (2016) EpiRADseq: scalable analysis of genomewide patterns of methylation using next-generation sequencing. Methods Ecol Evol 7:60–69

Schmitz RJ, Schultz MD, Lewsey MG, O'Malley RC, Urich MA, Libiger O, Schork NJ, Ecker JR (2011) Transgenerational epigenetic instability is a source of novel methylation variants. Science 334:369–373

Schnable PS, Springer NM (2013) Progress toward understanding heterosis in crop plants. Annu Rev Plant Bio l64:71–88

Schopfer CR, Nasrallah ME, Nasrallah JB (1999) The male determinant of self-incompatibility in *Brassica*. Science 286:1697–1700

Schranz ME, Quijada P, Sung SB, Lukens L, Amasino R, Osborn TC (2002) Characterization and effects of the replicated flowering time gene *FLC* in *Brassica rapa*. Genetics 162:1457–1468

Searle I, He Y, Turck F, Vincent C, Fornara F, Kröber S, Amasino RA, Coupland G (2006) The transcription factor FLC confers a flowering response to vernalization by repressing meristem competence and systemic signaling in *Arabidopsis*. Genes Dev 20:898–912

Seo PJ, Park MJ, Lim MH, Kim SG, Lee M, Baldwin IT, Park CM (2012) A self-regulatory circuit of CIRCADIAN CLOCK-ASSOCIATED1 underlies the circadian clock regulation of temperature responses in *Arabidopsis*. Plant Cell 24:2427–2442

Shah ZH, Rehman HM, Akhtar T, Daur I, Nawaz MA, Ahmad MQ, Rana IA, Atif RM, Yang SH, Chung G (2017) Redox and ionic homeostasis regulations against oxidative, salinity and drought stress in wheat (A Systems Biology Approach). Front Genet 8:1–10

Shan Q, Wang Y, Li J, Zhang Y, Chen K, Liang Z, Zhang K, Liu J, Xi JJ, Qiu JL, Gao C (2013a) Targeted genome modification of crop plants using a CRISPR-Cas system. Nat Biotechnol 31:686–688

Shan X, Wang X, Yang G, Wu Y, Su S, Li S, Liu H, Yuan Y (2013b) Analysis of the DNA methylation of maize (*Zea mays* L.) in response to cold stress based on methylation-sensitive amplified polymorphisms. J Plant Biol 56:32–38

Shanmugam A, Thamilarasan SG, Park JI, Jung MY, Nou IS (2016) Characterization and abiotic stress-responsive expression analysis of *SGT1*genesin *Brassica oleracea*. Genome 59:243–251

Sharma BB, Kalia P, Yadava DK, Singh D, Sharma TR (2016) Genetics and molecular mapping of black rot resistance locus *Xca1Bc* on chromosome B-7 in Ethiopian mustard (*Brassica carinata* A. Braun). PLoS One 11:e0152290

Shea DJ, Tomaru Y, Itabashi E, Nakamura Y, Miyazaki T, Kakizaki T, Naher TN, Shimizu M, Fujimoto R, Fukai E, Okazaki K (2018a) The production and characterization of a *BoFLC2* introgressed *Brassica rapa* by repeated backcrossing to an F_1. Breed Sci 68:316–325

Shea DJ, Itabashi E, Takada S, Fukai E, Kakizaki T, Fujimoto R, Okazaki K (2018b) The role of *FLOWERING LOCUSC* in vernalization of *Brassica*: the importance of vernalization research in the face of climate change. Crop Pasture Sci 69:30–39

Shea DJ, Shimizu M, Nishida N, Fukai E, Abe T, Fujimoto R, Okazaki K (2017) IntroMap: a signal analysis based method for the detection of genomic introgressions. BMC Genet 18:101

Shea DJ, Shimizu M, Itabashi E, Miyaji N, Miyazaki J, Osabe K, Kaji M, Okazaki K, Fujimoto R (2018c) Genome re-sequencing, SNP analysis, and genetic mapping of the parental lines of a commercial F_1 hybrid cultivar of Chinese cabbage. Breed Sci 68:375–380

Sheldon CC, Burn JE, Perez PP, Metzger J, Edwards JA, Peacock WJ, Dennis ES (1999) The *FLF* MADS box gene: a repressor of flowering in Arabidopsis regulated by vernalization and methylation. Plant Cell 11:445–458

Sheldon CC, Conn AB, Dennis ES, Peacock WJ (2002) Different regulatory regions are required for the vernalization-induced repression of *FLOWERING LOCUS C* and for the epigenetic maintenance of repression. Plant Cell 14:2527–2537

Shen H, He H, Li J, Chen W, Wang X, Guo L, Peng Z, He G, Zhong S, Qi Y, Terzaghi W, Deng XW (2012) Genome-wide analysis of DNA methylation and gene expression changes in two Arabidopsis ecotypes and their reciprocal hybrids. Plant Cell 24:875–892

Shen Y, Diener AC (2013) *Arabidopsis thaliana* RESISTANCE TO FUSARIUM OXYSPORUM 2 implicates tyrosine-sulfated peptide signaling in susceptibility and resistance to root infection. PLoS Genet 9:e1003525

Shen Y, Sun S, Hua S, Shen E, Ye CY, Cai D, Timko MP, Zhu QH, Fan L (2017) Analysis of transcriptional and epigenetic changes in hybrid vigor of allopolyploid *Brassica napus* uncovers key roles for small RNAs. Plant J 91:874–893

Sherf AF, MacNab AA (1986) Vegetable diseases and their control. John Wiley & Sons

Shi J, Li R, Zou J, Long Y, Meng J (2011) A dynamic and complex network regulates the heterosis of yield-correlated traits in rapeseed (*Brassica napus* L.). PLoS One 6:e21645

Shiba H, Iwano M, Entani T, Ishimoto K, Shimosato H, Che FS, Satta Y, Ito A, Takada Y, Watanebe M, Isogai A, Takayama S (2002) The dominance of alleles controlling self-incompatibility in *Brassica* pollen is regulated at the RNA level. Plant Cell 14:491–504

Shiba H, Kakizaki T, Iwano M, Tarutani Y, Watanabe M, Isogai A, Takayama S (2006) Dominance relationships between self-incompatibility alleles controlled by DNA methylation. Nat Genet 38:297–299

Shimizu M, Fujimoto R, Ying H, Pu Z, Ebe Y, Kawanabe T, Saeki N, Taylor JM, Kaji M, Dennis ES, Okazaki K (2014) Identification of candidate genes for fusarium yellows resistance in Chinese cabbage by differential expression analysis. Plant Mol Biol 85:247–257

Shimizu M, Pu Z, Kawanabe T, Kitashiba H, Matsumoto S, Ebe Y, Sano M, Funaki T, Fukai E, Fujimoto R, Okazaki K (2015) Map-based cloning of a candidate gene conferring Fusarium yellows resistance in *Brassica oleracea*. Theor Appl Genet 128:119–130

Shinozaki K, Yamaguchi-Shinozaki K (2000) Molecular responses to dehydration and low temperature: Differences and cross-talk between two stress signaling pathways. Curr Opin Plant Biol 3:217–223

Shinozaki K, Yamaguchi-Shinozaki K (2007) Gene networks involved in drought stress response and tolerance. J Exp Bot 58:221–227

Shopan J, Mou H, Zhang L, Zhang C, Ma W, Walsh JA, Hu Z, Yang J, Zhang M (2017) *Eukaryotic translation initiation factor 2B-beta* (*eIF2Bβ*), a new class of plant virus resistance gene. Plant J 90:929–940

Shull GH (1948) Whatis "heterosis"? Genetics 33:439–446

Singer T, Yordan C, Martienssen RA (2001) Robertson's *Mutator* transposons in *A. thaliana* are regulated by the chromatin-remodeling gene *Decrease in DNA Methylation(DDM1)*. Genes Dev 15:591–602

Singh D, Dhar S, Yadava DK (2011) Genetic and pathogenic variability of Indian strains of *Xanthomonas campestris* pv. *campestris* causing black rot disease in crucifers. Curr Microbiol 63:551–560

Singh D, Rathaur PS, Vicente JG (2016) Characterization, genetic diversity and distribution of *Xanthomonas campestris* pv. *campestris* races causing black rot disease in cruciferous crops of India. Plant Pathol 65:1411–1418

Smith KM (1935) A virus disease of cultivated crucifers. Ann Appl Biol 22:239–242

Soengas P, Hand P, Vicente JG, Pole JM, Pink DAC (2007) Identification of quantitative trait loci for resistance to *Xanthomonas campestris* pv. *campestris* in *Brassica rapa*. Theor Appl Genet 114:637–645

Song GS, Zhai HL, Peng YG, Zhang L, Wei G, Chen XY, Xiao YG, Wang L, Chen YJ, Wu B, Chen B, Zhang Y, Chen H, Feng XJ, Gong WK, Liu Y, Yin ZJ, Wang F, Liu GZ, Xu HL, Wei XL, Zhao XL, Ouwerkerk PB, Hankemeier T, Reijmers T, van der Heijden R, Lu CM, Wang M, van der Greef J, Zhu Z (2010) Comparative transcriptional profiling and preliminary study on heterosis mechanism of super-hybrid rice. Mol Plant 3:1012–1025

Song J, Angel A, Howard M, Dean C (2012) Vernalization–a cold-induced epigenetic switch. J Cell Sci 125:3723–3731

Song T, Chu M, Lahlali R, Yu F, Peng G (2016a) Shotgun label-free proteomic analysis of clubroot (*Plasmodiophora brassicae*) resistance conferred by the gene *Rcr1* in *Brassica rapa*. Front Plant Sci 7:1013

Song X, Liu G, Huang Z, Duan W, Tan H, Li Y, Hou X (2016b) Temperature expression patterns of genes and their coexpression with LncRNAs revealed by RNA-Seq in non-heading Chinese cabbage. BMC Genom 17:297

Song YH, Ito S, Imaizumi T (2013) Flowering time regulation: photoperiod- and temperature-sensing in leaves. Trends Plant Sci 18:575–583

Song YH, Shim JS, Kinmonth-Schultz HA, Imaizumi T (2015) Photoperiodic flowering: time measurement mechanisms in leaves. Annu Rev Plant Biol 66:441–464

Springer NM, Stupar RM (2007) Allelic variation and heterosis in maize: how do two halves make more than a whole? Genome Res 17:264–275

Srikanth A, Schmid M (2011) Regulation of flowering time: all roads lead to Rome. Cell Mol Life Sci 68:2013–2037

Staub T, Williams PH (1972) Factors influencing black rot lesion development in resistant and susceptible cabbage. Phytopathology 62:722–728

Stein JC, Howlett B, Boyes DC, Nasrallah ME, Nasrallah JB (1991) Molecular cloning of a putative receptor protein kinase gene encoded at the self-incompatibility locus of *Brassicaoleracea*. Proc Natl Acad Sci USA 88:8816–8820

Stief A, Altmann S, Hoffmann K, Pant BD, Scheible W, Bäurle I (2014) *Arabidopsis* miR156 regulates tolerance to recurring environmental stress through *SPL* transcription factors. Plant Cell 26:1792–1807

Stroud H, Do T, Du J, Zhong X, Feng S, Johnson L, Patel DJ, Jacobsen SE (2014) Non-CG methylation patterns shape the epigenetic landscape in *Arabidopsis*. Nat Struct Mol Biol 21:64–72

Suh SK, Green SK, Park HG (1995) Genetics of resistance to five strains of turnip mosaic virus in Chinese cabbage. Euphytica 81:71–77

Sung S, Amasino RM (2004) Vernalization in *Arabidopsis thaliana* is mediated by the PHD finger protein VIN3. Nature 427:159–164

Sung S, He Y, Eshoo TW, Tamada Y, Johnson L, Nakahigashi K, Goto K, Jacobsen SE, Amasino RM (2006) Epigenetic maintenance of the vernalized state in *Arabidopsis thaliana* requires LIKE HETEROCHROMATIN PROTEIN 1. Nat Genet 38:706–710

Suwabe K, Iketani H, Nunome T, Kage T, Hirai M (2002) Isolation and characterization of microsatellites in *Brassica rapa* L. Theor Appl Genet 104:1092–1098

Suwabe K, Tsukazak H, Iketani H, Hatakeyama K, Kondo M, Fujimura M, Nunome T, Fukuoka H, Hirai M, Matsumoto S (2006) Simple sequence repeat-based comparative genomics between *Brassica rapa* and *Arabidopsis thaliana*: the genetic origin of clubroot resistance. Genetics 173:309–319

Suwabe K, Tsukazaki H, Iketani H, Hatakeyama K, Fujimura M, Nunome T, Fukuoka H, Matsumoto S, Hirai M (2003) Identification of two loci for resistance to clubroot (*Plasmodiophora brassicae* Woronin) in *Brassica rapa* L. Theor Appl Genet 107:997–1002

Suzuki G, Kai N, Hirose T, Fukui K, Nishio T, Takayama S, Isogai A, Watanabe M, Hinata K (1999) Genomic organization of the *S* locus: Identification and characterization of genes in *SLG*/*SRK* region of S^9haplotype of *Brassicacampestris* (syn. *rapa*). Genetics 153:391–400

Swiezewski S, Liu F, Magusin A, Dean C (2009) Cold-induced silencing by long antisense transcripts of an *Arabidopsis* Polycomb target. Nature 462:799–802

Takahashi S, Fukushima N, Osabe K, Itabashi E, Shimizu M, Miyaji N, Takasaki-Yasuda T, Suzuki Y, Seki M, Fujimoto R (2018a) Identification of DNA methylated regions by using methylated DNA immunoprecipitation sequencing in *Brassica rapa*. Crop Pasture Sci 69:107–120

Takahashi S, Osabe K, Fukushima N, Takuno S, Miyaji N, Shimizu M, Takasaki-Yasuda T, Suzuki Y, Dennis ES, Seki M, Fujimoto R (2018b) Genome-wide characterization of DNA methylation, small RNA expression, and histone H3 lysine nine di-methylation in *Brassica rapa* L. DNA Res 25:511–520

Takasaki T, Hatakeyama K, Suzuki G, Watanabe M, Isogai A, Hinata K (2000) The *S* receptor kinase determines self-incompatibility in *Brassica* stigma. Nature 403:913–916

Takata K, Matsuzaki T, Tajika Y (2004) Aquaporins: Water channel proteins of the cell membrane. Prog Histochem Cytochem 39:1–83

Takayama S, Shimosato H, Shiba H, Funato M, Che FS, Watanabe M, Iwano M, Isogai A (2001) Direct ligand-receptor complex interaction controls *Brassica* self-incompatibility. Nature 413:535–538

Takuno S, Fujimoto R, Sugimura T, Sato K, Okamoto S, Zhang SL, Nishio T (2007) Effects of recombination on hitchhiking diversity in the Brassica self-incompatibility locus complex. Genetics 177:949–958

Tarutani Y, Shiba H, Iwano M, Kakizaki T, Suzuki G, Watanabe M, Isogai A, Takayama S (2010) *Trans*-acting small RNA determines dominance relationships in *Brassica* self-incompatibility. Nature 466:983–986

Taylor JD, Conway J, Roberts SJ, Astley D, Vicente JG (2002) Sources and origin of resistance to *Xanthomonas campestris* pv. *campestris* in *Brassica* genomes. Phytopathology 92:105–111

Teixeira FK, Heredia F, Sarazin A, Roudier F, Boccara M, Ciaudo C, Cruaud C, Poulain J, Berdasco M, Fraga MF, Voinnet O, Wincker P, Esteller M, Colot V (2009) A role for RNAi in the selective correction of DNA methylation defects. Science 323:1600–1604

Teutonico RA, Osborn TC (1994) Mapping of RFLP and qualitative trait loci in *Brassica rapa* and comparison to the linkage maps of *B. napus, B. oleracea,* and *Arabidopsis thaliana*. Theor Appl Genet 89:885–894

Thompson KF, Taylor JP (1966) Non-linear dominance relationships between *S*-alleles. Heredity 21:345–362

Tisdale WB (1923) Influence of soil temperature and soil moisture upon the Fusarium disease in cabbage seedlings. J Agric Res 24:55–86

Tomimura K, Špak J, Katis N, Jenner CE, Walsh JA, Gibbs AJ, Ohshima K (2004) Comparisons of the genetic structure of populations of *Turnip mosaic virus* in West and East Eurasia. Virology 330:408–423

Tomita H, Shimizu M, Doullah MA, Fujimoto R, Okazaki K (2013) Accumulation of quantitative trait loci conferring broad-spectrum clubroot resistance in *Brassica oleracea*. Mol Breed 32:889–900

Tomitaka Y, Ohshima K (2006) A phylogeographical study of the *Turnip mosaic virus* population in East Asia reveals an 'emergent' lineage in Japan. Mol Ecol 15:4437–4457

Tomlinson JA (1987) Epidemiology and control of virus disease of vegetables. Ann Appl Biol 110:661–681

Tonosaki K, Osabe K, Kawanabe T, Fujimoto R (2016) The importance of reproductive barriers and the effect of allopolyploidization on crop breeding. Breed Sci 66:333–349

Tonu NN, Doullah MA, Shimizu M, Karim MM, Kawanabe T, Fujimoto R, Okazaki K (2013) Comparison of positions of QTLs conferring resistance to *Xanthomonas campestris* pv. *campestris* in *Brassica oleracea*. Amer J Plant Sci 4:11–20

Tortosa M, Cartea ME, Rodríguez VM, Velasco P (2018) Unraveling the metabolic response of *Brassica oleracea* exposed to *Xanthomonas campestris* pv. *campestris*. J Sci Food Agri 98:3675–3683

Toxopeus H, Janssen AMP (1975) Clubroot resistance in turnip II. The 'slurry' screening method and clubroot races in the Netherlands. Euphytica 24:751–755

Tsai MC, Manor O, Wan Y, Mosammaparast N, Wang JK, Lan F, Shi Y, Segal E, Chang HY (2010) Long noncoding RNA as modular scaffold of histone modification complexes. Science 329:689–693

Tseng MJ, Liu CW, Yiu JC (2007) Enhanced tolerance to sulfur dioxide and salt stress of transgenic Chinese cabbage plants expressing both superoxide dismutase and catalase in chloroplasts. Plant Physiol Biochem 45:822–833

Tsukahara S, Kobayashi A, Kawabe A, Mathieu O, Miura A, Kakutani T (2009) Bursts of retrotransposition reproduced in *Arabidopsis*. Nature 461:423–426

Turck F, Fornara F, Coupland G (2008) Regulation and identity of florigen: *FLOWERING LOCUS T* moves center stage. Annu Rev Plant Biol 59:573–594

Turck F, Roudier F, Farrona S, Martin-Magniette ML, Guillaume E, Buisine N, Gagnot S, Martienssen RA, Coupland G, Colot V (2007) Arabidopsis TFL2/LHP1 specifically associates with genes marked by trimethylation of histone H3 lysine 27. PLoS Genet 3:e86

Turnbull C (2011) Long-distance regulation of flowering time. J Exp Bot 62:4399–4413

U N (1935) Genome analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. Jap J Bot 7:389–452

Ueno H, Matsumoto E, Aruga D, Kitagawa S, Matsumura H, Hayashida N (2012) Molecular characterization of the *CRa* gene conferring clubroot resistance in *Brassica rapa*. Plant Mol Biol 80:621–629

Ulloa M, Hutmacher RB, Davis RM, Wright SD, Percy R, Marsh B (2006) Breeding for Fusarium wilt race 4 resistance in cotton under field and greenhouse conditions. J Cotton Sci 10:114–127

Verma SS, Rahman MH, Deyholos MK, Basu U, Kav NNV (2014) Differential expression of miRNAs in *Brassica napus* root following infection with *Plasmodiophora brassicae*. PLoS ONE 9:e86648

Vicente JG, Conway J, Roberts SJ, Taylor JD (2001) Identification and origin of *Xanthomonas campestris* pv. *campestris* races and related pathovars. Phytopathology 91:492–499

Vicente JG, Holub EB (2013) *Xanthomonas campestris* pv. *campestris* (cause of black rot of crucifers) in the genomic era is still a worldwide threat to brassica crops. Mol Plant Pathol 14:2–18

Vicente JG, Taylor JD, Sharpe AG, Parkin IAP, Lydiate DJ, King GJ (2002) Inheritance of race-specific resistance to *Xanthomonas campestris* pv. *campestris* in *Brassica* genomes. Phytopathology 92:1134–1141

Vidalis A, Živković D, Wardenaar R, Roquis D, Tellier A, Johannes F (2016) Methylome evolution in plants. Genome Biol 17:264

Vongs A, Kakutani T, Martienssen RA, Richards EJ (1993) *Arabidopsis thaliana* DNA methylation mutants. Science 260:1926–1928

Voorrips RE (1995) *Plasmodiophora brassicae*: aspects of pathogenesis and resistance in *Brassica oleracea*. Euphytica 83:139–146

Voorrips RE, Jongerious MC, Kanne HJ (1997) Mapping of two genes for resistance to clubroot (*Plasmodiophora brassicae*) in a population of doubled haploid lines of *Brassica oleracea* by means of RFLP and AFLP markers. Theor Appl Genet 94:75–82

Voorrips RE, Kanne HJ (1997) Genetic analysis of resistance to clubroot (*Plasmodiophora brassicae*) in *Brassica oleracea.* I. Analysis of symptom grades. Euphytica 93:31–39

Walker JC (1930) Inheritance of Fusarium resistance in cabbage. J Agri Res 40:721–745

Walker JC (1933) Yellows-resistant lines of Early Jersey Wakefield cabbage. J Agri Res 46:639–648

Walker JC, Blank LM (1934) Fusarium resistant Danish ballhead cabbage. J Agri Res 49:983–989

Walker JC, Monteith JJ, Wellman FL (1927) Development of three midseason varieties of cabbage resistant to yellows (*Fusarium conglutinans* Woll.). J Agric Res 35:785–809

Walsh JA (1989) Genetic control of immunity to turnip mosaic virus in winter oilseed rape (*Brassica napus* ssp. *oleifera*) and the effect of foreign isolates of the virus. Ann Appl Biol 115:89–99

Walsh JA, Jenner CE (2002) *Turnip mosaic virus* and the quest for durable resistance. Mol Plant Pathol 3:289–300

Walsh JA, Rusholme RL, Hughes SL, Jenner CE, Bambridge JM, Lydiate DJ, Green SK (2002) Different classes of resistance to Turnip mosaic virus in *Brassica rapa*. Eur J Plant Pathol 108:15–20

Walsh JA, Sharpe AG, Jenner CE, Lydiate DJ (1999) Characterisation of resistance to turnip mosaic virus in oilseed rapa (*Brassica napus*) and genetic mapping of TuRB01. Theor Appl Genet 99:1149–1154

Wang X, Wang H, Wang J, Sun R, Wu J, Liu S, Bai Y, Mun JH, Bancroft I, Cheng F, Huang S, Li X, Hua W, Wang J, Wang X, Freeling M, Pires JC, Paterson AH, Chalhoub B, Wang B, Hayward A, Sharpe AG, Park BS, Weisshaar B, Liu B, Li B, Liu B, Tong C, Song C, Duran C, Peng C, Geng C, Koh C, Lin C, Edwards D, Mu D, Shen D, Soumpourou E, Li F, Fraser F, Conant G, Lassalle G, King GJ, Bonnema G, Tang H, Wang H, Belcram H, Zhou H, Hirakawa H, Abe H, Guo H, Wang H, Jin H, Parkin IAP, Batley J, Kim JS, Just J, Li J, Xu J, Deng J, Kim JA, Li J, Yu J, Meng J, Wang J, Min J, Poulain J, Wang J, Hatakeyama K, Wu K, Wang L, Fang L, Trick M, Links MG, Zhao M, Jin M, Ramchiary N, Drou N, Berkman PJ, Cai Q, Huang Q, Li R, Tabata S, Cheng S, Zhang S, Zhang S, Huang S, Sato S, Sun S, Kwon SJ, Choi SR, Lee TH, Fan W, Zhao X, Tan X, Xu X, Wang Y, Qiu Y, Yin Y, Li Y, Du Y, Liao Y, Lim Y, Narusaka Y, Wang Y, Wang Z, Li Z, Wang Z, Xiong Z, Zhang Z (2011) The genome of the mesopolyploid crop species *Brassica rapa*. Nat Genet 43:1035–1039

Wang ZY, Kenigsbuch D, Sun L, Harel E, Ong MS, Tobin EM (1997) A Myb-related transcription factor is involved in the phytochrome regulation of an Arabidopsis *Lhcb* gene. Plant Cell 9:491–507

Wang ZY, Tobin EM (1998) Constitutive expression of the *CIRCADIAN CLOCK ASSOCIATED 1 (CCA1)* gene disrupts circadian rhythms and suppresses its own expression. Cell 93:1207–1217

Warwick SI, Francis A, Al-Shehbaz IA (2006) Brassicaceae: species checklist and database on CD-Rom. Plant Syst Evol 259:249–258

Wasilewska A, Vlad F, Sirichandra C, Redko Y, Jammes F, Valon C, Frei dit Fery N, Leung J (2008) An update on abscisic acid signaling in plants and more. Mol Plant 1:198–217

Watanabe M, Ito A, Takada Y, Ninomiya C, Kakizaki T, Takahata T, Hatakeyama K, Hinata K, Suzuki G, Takasaki T, Satta Y, Shiba H, Takayama S, Isogai A (2000) Highly divergent sequences of the pollen self-incompatibility (*S*) gene in class-I *S* haplotypes of *Brassicacampestris* (syn. *rapa*) L. FEBS Lett 473:139–144

Watanabe M, Ono T, Hatakeyama K, Takayama S, Isogai A, Hinata K (1997) Molecular characterization of *SLG* and *S*-related genes in a self-compatible *Brassica campestris* L. var. *yellow sarson*. Sex Plant Reprod 10:332–340

Weigel D, Ahn JH, Blázquez MA, Borevitz JO, Christensen SK, Fankhauser C, Ferrándiz C, Kardailsky I, Malancharuvil EJ, Neff MM, Nguyen JT, Sato S, Wang ZY, Xia Y, Dixon RA, Harrison MJ, Lamb CJ, Yanofsky MF, Chory J (2000) Activation tagging in Arabidopsis. Plant Physiol 122:1003–1013

Werner S, Diederichsen E, Frauen M, Schondelmaier J, Jung C (2008) Genetic mapping of clubroot resistance genes in oilseed rape. Theor Appl Genet 116:363–372

Wigge PA, Kim MC, Jaeger KE, Busch W, Schmid M, Lohmann JU, Weigel D (2005) Integration of spatial and temporal information during floral induction in *Arabidopsis*. Science 309:1056–1059

Wit F, Van De Weg M (1964) Clubroot-resistance in turnips (*Brassica campestris* L.) I. Physiological races of the parasite and their identification in mixtures. Euphytica 13:9–18

Wittmann S, Chatel H, Fortin MG, Laliberté JF (1997) Interaction of the viral protein genome linked of Turnip mosaic potyvirus with the translational eukaryotic initiation factor (iso) 4E of *Arabidopsis thaliana* using the yeast two-hybrid system. Virol 234:84–92

Wood CC, Robertson M, Tanner G, Peacock WJ, Dennis ES, Helliwell CA (2006) The *Arabidopsis thaliana* vernalization response requires a polycomb-like protein complex that also includes VERNALIZATION INSENSITIVE 3. Proc Natl Acad Sci USA 103:14631–14636

Wu P, Wang W, Duan W, Li Y, Hou X (2017) Comprehensive analysis of the CDPK-SnRK superfamily genes in Chinese cabbage and its evolutionary implications in plants. Front Plant Sci 8:162

Xiao D, Zhao JJ, Hou XL, Basnet RK, Carpio DP, Zhang NW, Bucher J, Lin K, Cheng F, Wang XW, Bonnema G (2013) The *Brassica rapaFLC* homologue *FLC2* is a key regulator of flowering time, identified through transcriptional co-expression networks. J Exp Bot 64:4503–4516

Xiao J, Lee US, Wagner D (2016) Tug of war: adding and removing histone lysine methylation in *Arabidopsis*. CurrOpin Plant Biol 34:41–53

Xing M, Lv H, Ma J, Xu D, Li H, Yang L, Kang J, Wang X, Fang Z (2016) Transcriptome profiling of resistance to *Fusarium oxysporum* f. sp. *conglutinans* in cabbage (*Brassica oleracea*) roots. PLoS One 11:e0148048

Xu L, Ren L, Chen K, Liu F, Fang X (2016) Putative role of IAA during the early response of *Brassica napus* L. to *Plasmodiophora brassicae*. Eur J Plant Pathol 145:601–613

Yamagishi H, Bhat SR (2014) Cytoplasmic male sterility in Brassicaceae crops. Breed Sci 64:38–47

Yan L, Loukoianov A, Tranquilli G, Helguera M, Fahima T, Dubcovsky J (2003) Positional cloning of the wheat vernalization gene *VRN1*. Proc Natl Acad Sci USA 100:6263–6268

Yang J, Liu D, Wang X, Ji C, Cheng F, Liu B, Hu Z, Chen S, Pental D, Ju Y, Yao P, Li X, Xie K, Zhang J, Wang J, Liu F, Ma W, Shopan J, Zheng H, Mackenzie SA, Zhang M (2016) The genome sequence of allopolyploid *Brassica juncea* and analysis of differential homoeolog gene expression influencing selection. Nat Genet 50:1616

Yang L, Li B, Zheng XY, Li J, Yang M, Dong X, He G, An C, Deng XW (2015) Salicylic acid biosynthesis is enhanced and contributes to increased biotrophic pathogen resistance in *Arabidopsis* hybrids. Nat Commun 6:7309

Yang M, Wang X, Ren D, Huang H, Xu M, He G, Deng XW (2017a) Genomic architecture of biomass heterosis in *Arabidopsis*. Proc Natl Acad Sci USA 114:8101–8106

Yang Y, Zhu K, Li H, Han S, Meng Q, Khan SU, Fan C, Xie K, Zhou Y (2017b) Precise editing of *CLAVATA* genes in *Brassica napus* L. regulates multilocular silique development. Plant Biotechnol J 16:1322–1335

Yasuda S, Wada Y, Kakizaki T, Tarutani Y, Miura-Uno E, Murase K, Fujii S, Hioki T, Shimoda T, Takada Y, Shiba H, Takasaki-Yasuda T, Suzuki G, Watanabe M, Takayama S (2016) A complex

dominance hierarchy is controlled by polymorphism of small RNAs and their targets. Nat Plants 3:16206

Yeam I, Cavatorta JR, Ripoll DR, Kang BC, Jahn MM (2007) Functional dissection of naturally occurring amino acid substitutions in eIF4E that confers recessive potyvirus resistance in plants. Plant Cell 19:2913–2928

Yin Z, Rorat T, Szabala BM, Ziółkowska A, Malepszy S (2006) Expression of a *Solanum sogarandinum* SK3-type dehydrin enhances cold tolerance in transgenic cucumber seedlings. Plant Sci 170:1164–1172

Yoon JY, Green SK, Opeña RT (1993) Inheritance of resistance to turnip mosaic virus in Chinese cabbage. Euphytica 69:103–108

Yu F, Zhang X, Huang Z, Chu M, Song T, Falk KC, Deora A, Chen Q, Zhang Y, McGregor L, Gossen BD, McDonald MR, Peng G (2016) Identification of genome-wide variants and discovery of variants associated with *Brassica rapa* clubroot resistance gene *Rcr1* through bulked segregant RNA sequencing. PLoS ONE 11:e0153218

Yu F, Zhang X, Peng G, Falk KC, Strelkov SE, Gossen BD (2017) Genotyping-by-sequencing reveals three QTL for clubroot resistance to six pathotypes of *Plasmodiophora brassicae* in *Brassica rapa*. Sci Rep 7:4516

Yu Q, Hu Y, Li J, Wu Q, Lin Z (2005) Sense and antisense expression of plasma membrane aquaporin *BnPIP1* from *Brassica napus* in tobacco and its effects on plant drought resistance. Plant Sci 169:647–656

Yu X, Wang H, Lu Y, de Ruiter M, Cariaso M, Prins M, van Tunen A, He Y (2012) Identification of conserved and novel microRNAs that are responsive to heat stress in *Brassica rapa*. J Exp Bot 63:1025–1038

Yu X, Yang J, Li X, Liu X, Sun C, Wu F, He Y (2013) Global analysis of cis-natural antisense transcripts and their heat-responsive nat-siRNAs in *Brassica rapa*. BMC Plant Biol 13:208

Zemach A, Kim MY, Hsieh PH, Coleman-Derr D, Eshed-Williams L, Thao K, Harmer SL, Zilberman D (2013) The *Arabidopsis* nucleosome remodeler DDM1 allows DNA methyltransferases to access H1-containing heterochromatin. Cell 153:193–205

Zemach A, McDaniel IE, Silva P, Zilberman D (2010) Genome-wide evolutionary analysis of eukaryotic DNA methylation. Science 328:916–919

Zhang J, Lu Y, Yuan Y, Zhang X, Geng J, Chen Y, Cloutier S, McVetty PB, Li G (2008) Map-based cloning and characterization of a gene controlling hairiness and seed coat color traits in *Brassica rapa*. Plant Mol Biol 5:553–563

Zhang Q, Li Y, Xu T, Srivastava AK, Wang D, Zeng L, Yang L, He L, Zhang H, Zheng Z, Yang DL, Zhao C, Dong J, Gong Z, Liu R, Zhu JK (2016a) The chromatin remodeler DDM1 promotes hybrid vigor by regulating salicylic acid metabolism. Cell Discov 2:16027

Zhang T, Zhao Z, Zhang C, Pang W, Choi SR, Lim YP, Piao Z (2014) Fine genetic and physical mapping of the *CRb* gene conferring resistance to clubroot disease in *Brassica rapa*. Mol Breed 34:1173–1183

Zhang X, Bernatavichute YV, Cokus S, Pellegrini M, Jacobsen SE (2009) Genome-wide analysis of mono-, di-and trimethylation of histone H3 lysine 4 in *Arabidopsis thaliana*. Genome Biol 10:R62

Zhang X, Clarenz O, Cokus S, Bernatavichute YV, Pellegrini M, Goodrich J, Jacobsen SE (2007) Whole-genome analysis of histone H3 lysine 27 trimethylation in Arabidopsis. PLoS Biol 5:e129

Zhang X, Liu Y, Fang Z, Li Z, Yang L, Zhuang M, Zhang Y, Lv H (2016b) Comparative transcriptome analysis between broccoli (*Brassica oleracea* var. *italica*) and wild cabbage (*Brassica macrocarpa* Guss.) in response to *Plasmodiophora brassicae* during different infection stages. Front Plant Sci 7:1929

Zhang X, Meng L, Liu B, Hu Y, Cheng F, Liang J, Aarts MG, Wang X, Wu J (2015) A transposon insertion in *FLOWERING LOCUS T* is associated with delayed flowering in *Brassica rapa*. Plant Sci 241:211–220

Zhang X, Yazaki J, Sundaresan A, Cokus S, Chan SW, Chen H, Henderson IR, Shinn P, Pellegrini M, Jacobsen SE, Ecker JR (2006) Genome-wide high-resolution mapping and functional analysis of DNA methylation in *Arabidopsis*. Cell 126:1189–1201

Zhao J, Kulkarni V, Liu N, Pino Del Carpio D, Bucher J, Bonnema G (2010) *BrFLC2 (FLOWERING LOCUS C*) as a candidate gene for a vernalization response QTL in *Brassica rapa*. J Exp Bot 61:1817–1825

Zhen G, Qin P, Liu KY, Nie DY, Yang YZ, Deng XW, He H (2017) Genome-wide dissection of heterosis for yield traits in two-line hybrid rice populations. Sci Rep 7:7635

Zheng B, Chen X (2011) Dynamics of histone H3 lysine 27 trimethylation in plant development. Curr Opin Plant Biol 14:123–129

Zhou S, Hu W, Deng X, Ma Z, Chen L, Huang C, Wang C, Wang J, He Y, Yang G, He G (2012) Overexpression of the wheat aquaporin gene, *TaAQP7*, enhances drought tolerance in transgenic tobacco. PLoS ONE 7:e52439

Zhu JK (2016) Abiotic stress signaling and responses in plants. Cell 167:313–324

Zhu M, Assmann SM (2017) Metabolic signatures in response to abscisic acid (ABA) treatment in *Brassica napus* guard cells revealed by metabolomics. Sci Rep 7:12875

Zhu QH, Stephen S, Kazan K, Jin G, Fan L, Taylor J, Dennis ES, Helliwell CA, Wang MB (2013) Characterization of the defense transcriptome responsive to *Fusarium oxysporum*-infection in *Arabidopsis* using RNA-seq. Gene 512:259–266

Zielinski RE (1998) Calmodulin and calmodulin-binding proteins in plants. Annu Rev Plant Physiol Plant Mol Biol 49:697–725

Zilberman D, Gehring M, Tran RK, Ballinger T, Henikoff S (2007) Genome-wide analysis of *Arabidopsis thaliana* DNA methylation uncovers an interdependence between methylation and transcription. Nat Genet 39:61–69

Chapter 4
Eggplant Breeding and Improvement for Future Climates

Mark A. Chapman

Abstract The Asian eggplant, *Solanum melongena* (also known as aubergine or brinjal), is a widely grown and economically important crop, especially in South and Southeast Asia and the Mediterranean. A large amount of morphological diversity is present in eggplant, suggesting that genetic variation is high; however, limited work has been carried out on traits which could be of importance in a future climate. Here I discuss what is known about biotic and abiotic tolerances in eggplant, and in particular highlight that the variation in the crop-wild relatives, found throughout Africa and Southern Asia, is likely to be very important for breeding eggplants for future climates. I also discuss the limited knowledge we currently have on two other domesticated eggplants, the scarlet eggplant (*S. aethiopicum* L.) and the Gboma eggplant (*S. macrocarpon* L.). The chapter ends with some considerations for future work, and I highlight that the development of introgression populations, the study and conservation of eggplant wild relatives, and the genetic dissection of adaptive traits should be prioritized.

Keywords Crop-wild relatives · Eggplant · Pathogen resistance · Pest resistance · Stress tolerance

4.1 Overview

Globally, with climate change, we expect increase in temperature and CO_2, as well as additional unpredictability with regard to droughts, floods, and storms (Coumou and Rahmstorf 2012; Trenberth et al. 2013; Poppy et al. 2014), and this is occurring at the same time as the world population is dramatically increasing in size (Godfray et al. 2010). Increasing temperatures are predicted to have a significant effect on crop yields (Zhao et al. 2017) and the rapid development of novel tolerant varieties is required to counteract this (Challinor et al. 2016; Atlin et al. 2017).

With this increasing world population, efforts need to be made to produce more food and utilizing sub-optimal land (Tilman et al. 2002). It is clearly important to

M. A. Chapman (✉)
Biological Sciences, University of Southampton, SO17 1BJ Southampton, UK
e-mail: m.chapman@soton.ac.uk

C. Kole (ed.), *Genomic Designing of Climate-Smart Vegetable Crops*,
https://doi.org/10.1007/978-3-319-97415-6_4

increase crop yields; however, if we wish to expand the growing areas of crops, we need to develop varieties with ability to tolerate poor soils, more erratic precipitation, and/or salinity (Ahuja et al. 2010).

Coupled with the immediate abiotic pressures of climate change and poor growing conditions, future crops may have to tolerate novel suites of pests and pathogens. It is estimated that crop yields are reduced by 20–40% due to these biotic pressures (e.g., Oerke et al. 1994), and with climate change it is predicted that the natural geographic ranges of many pathogens will change (Garrett et al. 2006; Savary et al. 2012). This additional (and currently somewhat unpredictable; Donatelli et al. 2017) pressure, novel pests, and diseases could result in a significant drop in yield even if climate-tolerant varieties are developed.

Asian eggplant (*S. melongena*) is an important vegetable crop, especially in parts of the Mediterranean, the Middle East, and Southern/Southeastern Asia. It is the third most widely grown crop in the Solanaceae, after tomato and potato, grown on about 1.86 million (M) ha and with a total production of 52.3 M tons globally in 2017 (FAO 2017). The majority of eggplant (over 80%) is grown in China and India. India in particular is at extreme threat from climate change, with the expectation of increased temperatures, varied precipitation patterns, and unpredictability of the monsoons that millions of farmers rely on (World Bank 2013). Two-thirds of agriculture in India is rain-fed; therefore, >250 M farmers and associated landless agricultural laborers currently rely on the monsoon.

Adaptation of eggplant to the future climate could be achieved through (1) the identification of stress-tolerant eggplant varieties, (2) the breeding of eggplant with stress-tolerant wild relatives, and/or (3) mutational/transgenic approaches. These will be discussed in turn in this review, after an introduction to the Asian eggplant.

While the focus of this chapter is the Asian eggplant, two other eggplant species can be described as domesticated; these are the Ethiopian or scarlet eggplant (*S. aethiopicum* L.) and the African or Gboma eggplant (*S. macrocarpon* L.). Less research has been carried out on these other eggplants (in general, as well as specifically looking at climatic tolerances); however, the research that is available is reviewed in Sect. 4.6.

4.2 Origin of Asian Eggplant and Phylogenetic Relationships to Wild Relatives

Eggplant was domesticated in the Old World, in contrast to its New World congeners tomato and potato, most likely in or near to present-day Malaysia, Thailand, and Vietnam (Page et al. 2019b). Enormous morphological variation exists in the eggplant gene pool, most notably in fruit size, shape, and color characteristics, and also in plant stature, length of growing period, and flower shape and color. The wild progenitor is *Solanum insanum* L., a widespread species found as far West as Madagascar,

extending throughout India to Thailand, Indonesia, and Malaysia in the East (Ranil et al. 2017).

Recent analysis based on ca. 5,000 nuclear single nucleotide polymorphisms (SNPs) demonstrates that eggplants with fruit the shape and size of hen's eggs, previously named *S. ovigerum*, represent primitive domesticates, with further selection resulting in landraces with larger and more diverse shapes and sizes of fruits (Page et al. 2019b). The domestication bottleneck (the loss of diversity expected due to humans selecting only a subset of the genetic variation present in the wild) is estimated to have resulted in a ca. 50% reduction in genetic diversity in *S. melongena* relative to *S. insanum*. The cultivated species is roughly split into Eastern (Chinese, Thai, Indonesian, Filipino, and Malaysian) and Western (Indian) landrace groups based on the same panel of SNPs.

Several recent taxonomic works have provided more detail into the relationships between *S. melongena* and related wild eggplants. The eggplant clade, along with the wild relatives of *S. melongena* (Knapp et al. 2013), is a well-supported monophyletic group of 10–13 species (Vorontsova et al. 2013; Aubriot et al. 2018) distributed throughout Africa, the Middle East, and into Southeast Asia. It appears that the eggplant clade originated in the Middle East/Northeast Africa and then expanded into Africa (where most eggplant clade species are found) and into South Asia (where *S. insanum* is found and domestication took place) (Aubriot et al. 2018).

4.3 Climate Change-Relevant Genetic and Phenotypic Variation in Eggplant

In a review of the World Vegetable Center Eggplant Collection, Taher et al. (2017) highlight that while yield and fruit quality have been relatively well characterized, screening for biotic and abiotic stress tolerance has lagged behind. More generally, while the genetic basis of stress tolerance is being explored in a number of crops, there are scant examples of where this has been applied to breeding programs (Gilliham et al. 2017).

There are several pathogens which cause damage to eggplants, ranging from bacteria to fungi to insects. Screening investigations have identified accessions with the strongest resistance to Fusarium wilt (Boyaci et al. 2012), bacterial wilt (Daunay 2008; Lebeau et al. 2011), and *Ralstonia* (Daunay 2008; Salgon et al. 2018). Within the eggplant gene pool there also exist varieties resistant to leafhopper, aphids, and eggplant root and shoot borer (reviewed in Taher et al. 2017). Resistance to *Verticillium* wilt or root-knot nematodes (*Meloidogyne* spp.) has not been found (Daunay et al. in press), and the breeding of eggplants resistant to these latter pests relies on wild relatives (see next section). The genetic basis of resistance to Fusarium wilt has been determined based on crossing resistant and susceptible eggplant varieties (Mutlu et al. 2008; Boyaci et al. 2012; Miyatake et al. 2016). Similarly, resistance to *Ralstonia* has been mapped (Lebeau et al. 2013; Salgon et al. 2018).

Salt tolerance and chilling tolerance have been investigated in small numbers of cultivars (Minghua et al. 2001; Yasar 2003); however, drought tolerance in the eggplant gene pool appears to be understudied. In one eggplant cultivar, fruit production and leaf area are positively correlated with the number of lateral roots (Rouhani et al. 1987), which suggests that variation in root growth parameters is important to analyze. Recently, Bui et al. (2015) compared nine *S. melongena* accessions (and one *S. linnaeanum* Hepper and P.-M.L. Jaeger accession) for root traits thought to be correlated with drought tolerance. Rate of adventitious root emission showed considerable variation among the genotypes, which could play an adaptive role in adaptation to low water. Further, genotypes with high growth rate also had fast-growing densely branched roots (Bui et al. 2015) indicating that above-ground growth can be taken as a proxy for root growth without the need for extensive below-ground phenotyping.

While different varieties can have different levels of resistance to a stress, variation in pathogen resistance and stress tolerance can often be affected by external factors, for example, the presence of other stresses, a scenario likely to be encountered in the wild (Mittler 2006). These multiple stresses can give rise to synergistic or antagonistic responses, increased damage, or in some cases one stress can result in tolerance of a second stress. For example, tomato plants under drought conditions can be more resistant to fungal infection (Achuo et al. 2006) and *Arabidopsis* plants exposed to *Verticillium dahlia*, a fungal pathogen, demonstrated an increase in drought tolerance (Reusche et al. 2012).

In eggplant, simultaneous application of *Verticillium* infection and drought affected two eggplant cultivars in different ways, and not in the way as predicted by the effect of each stress applied individually. For example, *Verticillium* infection reduced relative growth rate (RGR) marginally in one cultivar under both control and drought conditions, whereas for the other cultivar a much greater reduction in RGR was observed under drought relative to control (Tani et al. 2018).

Other studies have examined the effect of the environment on trait expression. For example, phenolic compounds, common in eggplant fruit and with health benefits to the consumer, were significantly more abundant in spring-harvested than summer-harvested fruits (García-Salas et al. 2014), and in quantitative trait loci (QTL) mapping studies, some QTLs are found in only a subset of the environments in which the population is grown (e.g., Doganlar et al. 2002b; Toppino et al. 2016).

The examination of eggplant genotypes as a rootstock deserves investigation too. Eggplant is an important rootstock for a number of other crops because of tolerance to certain biotic and abiotic factors. In some countries more than half of the tomatoes produced are from plants which were grown on a rootstock (Lee et al. 2010), and eggplant is a commonly used rootstock. As an example, eggplants serve as a bacterial wilt-resistant rootstock for peppers and tomatoes (Sadashiva et al. 2001), and waterlogging tolerance is greater in tomato grafted onto eggplant rootstocks than in non-grafted tomato plants (Bahadur et al. 2015). Varieties typically used as rootstock have been identified based on tolerance in the current climate, but there appears to be no work analyzing these varieties under varying environmental pressures.

4.4 Climate Change-Relevant Genetic and Phenotypic Variation in Eggplant Wild Relatives

It has become evident that crop-wild relatives (CWRs) can contain adaptive genetic variation that is absent from domesticated crops (Maxted et al. 2007). This comes from the domestication bottleneck (i.e., only a subset of genetic variation present in the progenitor is found in the domesticated species), and also because each crop typically has many CWRs, likely to be found in diverse environments adapted to different selection pressures. Maxted and Kell (2009)reported that ca. 1,000 plant species can be considered CWRs very closely related to some of the world's most important food crops; however, 75% of these are threatened in the wild and/or poorly represented in gene banks (Dempewolf et al. 2014).

If the CWRs can be bred with the crop, then there is the potential for this novel variation to be crossed into the crop. In a relatively small number of generations, and enhanced by molecular breeding techniques, such as marker-assisted selection (MAS; Morrell et al. 2011), stress or pest tolerance from a wild species can be introgressed into a crop genetic background (Tanksley and McCouch 1997; Warschefsky et al. 2014). Hajjar and Hodgkin (2007) reported that the majority of CWR usage (ca. 80%) is for the crossing of pest and disease resistance into crops, highlighting how environmental tolerance was a low priority just a decade ago.

The success of breeding attempts between a crop and its wild relatives gives rise to the concept of gene pools (Harlan and de Wet 1971). The crop primary gene pool is expected to contain the wild progenitor species, which usually freely interbreeds with the crop. The secondary gene pool contains species which can be crossed to the crop, but exhibit partial reproductive isolation, for example, the crosses generate weak or partially sterile hybrids. The tertiary gene pool contains species which can only be crossed with the crop if embryo rescue or a bridging species is used. For eggplant, the primary gene pool contains only *S. insanum*, and the extent of free interbreeding between these two species is evidenced by several reports of gene flow in the wild (Davidar et al. 2015; Page et al. 2019b). The secondary gene pool of eggplant contains potentially 48 species (although taxonomic revision may change this number) and the tertiary gene pool only a handful (Syfert et al. 2016).

Eggplant is one of the 29 species prioritized by The Millennium Seed Bank (Royal Botanic Gardens, Kew) and the Global Crop Diversity Trust for the collection and conservation of CWRs (https://www.cwrdiversity.org/), highlighting the potential importance of CWRs toward the breeding of climate-resilient eggplant.

A number of eggplant CWRs have been identified with resistance to specific pests (reviewed in Kashyap et al. 2003; Syfert et al. 2016); however, in some cases, the generation of plants beyond the F_1 has proved difficult or impossible. For example, *S. sisymbriifolium* Lam. and *S. torvum* Sw. are resistant to *Ralstonia* and *Fusarium* wilts and root-knot nematodes (*Meloidogyne* spp.) and attempts have been made to cross these species with eggplant. In one case, F_1 hybrids between *S. sisymbriifolium* and *S. melongena* produced sterile seeds (Collonnier et al. 2003) and crosses between *S. torvum* and *S. melongena* could only be produced using embryo rescue (Bletsos

et al. 1998; Kumchai et al. 2013) or protoplast fusion (Jarl et al. 1999). Backcrosses between *S. torvum* x *S. melongena* F_1s and the parents were only possible when *S. melongena* was the male parent, and even then only some eggplant cultivars were successful fathers (Bletsos et al. 1998).

Better success has come from crossing two *Fusarium* wilt-resistant species, *S. incanum* L. (Lester and Kang 1998; Plazas et al. 2016) and *S. violaceum* Ortega (Rao and Kumar 1980, named *S. indicum* L. in their study), with *S. melongena*. The variable success of multiple attempts at the former cross, however, highlights how there can be extensive variability observed by different authors (reviewed in Daunay et al. 2019). Some successful crosses have been made between *S. linnaeanum*, a species with resistance to *Verticillium* wilt, and *S. melongena*; however, only one of four eggplant cultivars would successfully cross with *S. linnaeanum* (Liu et al. 2015).

While being less-studied than pest and pathogen resistance, the development of eggplant varieties with improved or novel environmental tolerances will be crucial to managing the risks associated with climate change. The large natural distribution of eggplant's progenitor, *Solanum insanum* (Ranil et al. 2017; Fig. 4.1), as well as other related species, for example, *S. campylacanthum*, suggests that different populations of these species are likely to be locally adapted (Knapp et al. 2013). Some

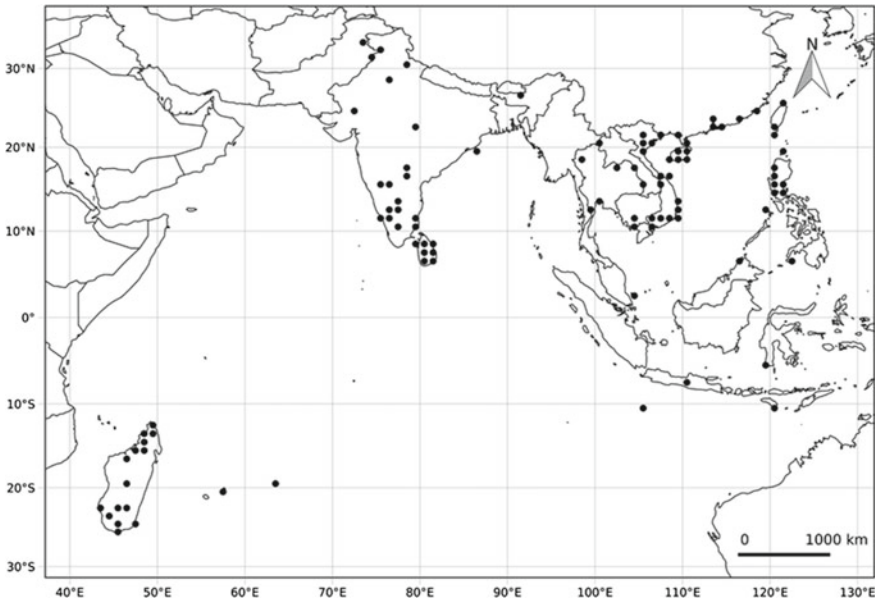

Fig. 4.1 Distribution of *S. insanum*, the wild progenitor of Asian eggplant, *S. melongena* according to herbarium collections observed by Ranil et al. (2017). Gaps in the distribution reflect gaps in collecting efforts and/or countries where herbaria have not been thoroughly examined. Figure taken from Ranil et al. (2017) under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/)

populations could therefore contain alleles at genes involved in adaptation to temperature, precipitation, and other stresses. Identifying adaptive variation in these species would be a significant first step in selecting material for breeding with eggplant. It is noteworthy that *S. campylacanthum* is a tetraploid, whereas *S. melongena* and many of the CWRs are diploid (Page et al. 2019b); hence, breeding attempts with *S. campylacanthum* might be met with initial sterility problems. Drought-tolerant *S. elaeagnifolium* (Christodoulakis et al. 2009; Fita et al. 2015) could not be crossed with eggplant by Plazas et al. (2016), but a single fruit with a small number of viable embryos was produced by Kouassi et al. (2016).

Significant advances have been made recently in regard to the detailed phenotyping of eggplant CWRs and (eggplant x CWR) F_1 hybrids (Kaushik et al. 2016; Fig. 4.2) and using these F_1 hybrids to generate backcross populations to eggplant (Kouassi et al. 2016). Although phenotyping of these crosses so far is limited to morphological descriptors with obvious agronomic benefits (for example, fruit size and shape), some of the traits might be of adaptive value in climate change scenarios (e.g., spininess/prickliness as herbivore deterrent and plant height under abiotic stress; Fig. 4.2).

The generation of backcross populations has utilized a range of CWRs, including the progenitor species *S. insanum*, as well as *S. anguivi*, *S. dasyphyllum*, *S. incanum*, *S. lichtensteinii*, and *S. tomentosum* (Kouassi et al. 2016). These species have several important traits which could be of adaptive value, including resistance to *Ralstonia* (*S. anguivi* [Schippers 2002]), drought tolerance (*S. incanum* [Daunay 2008; Lester and Hasan 1991] and *S. lichtensteinii* [Vorontsova and Knapp 2012]), and salinity tolerance/resistance to Verticillium wilt (*S. linnaeanum* [Daunay et al. 1991; Liu et al. 2015]). Genetic mapping in *S. linnaeanum* x *S. melongena* F2 populations has provided knowledge concerning the genetic basis of agronomic phenotypes (Doganlar et al. 2002b) and Verticillium resistance (Sunseri et al. 2003). Recombinant inbred lines (RILs) from the cross between *S. melongena* and *S. linnaeanum* used by Doganlar et al. (2002a, b) have been developed (M-C Brand-Daunay, personal communication).

An introgression line (IL) population is being developed in which *S. incanum* genome fragments are present in a *S. melongena* background, and preliminary phenotyping indicates that drought tolerance and other valuable traits are variable (Gramazio et al. 2017).

An analysis of drought tolerance (specifically the maintenance of growth under a 50% water deficit) in eggplant and CWRs was undertaken by Fita et al. (2015) in which nine eggplant varieties and six CWRs were investigated. One eggplant accession exhibited good tolerance to water deficit as did the tertiary gene pool species *S. elaeagnifolium*. Interestingly, tolerance was afforded by different mechanisms in different genotypes, intimating that crosses between these types could afford an even greater level of drought tolerance. Root vigor and plant architecture are also being investigated in *S. elaeagnifolium–S. melongena* crosses, with the potential for these traits to be linked to the observed drought tolerance (Garcia-Fortea et al. 2019).

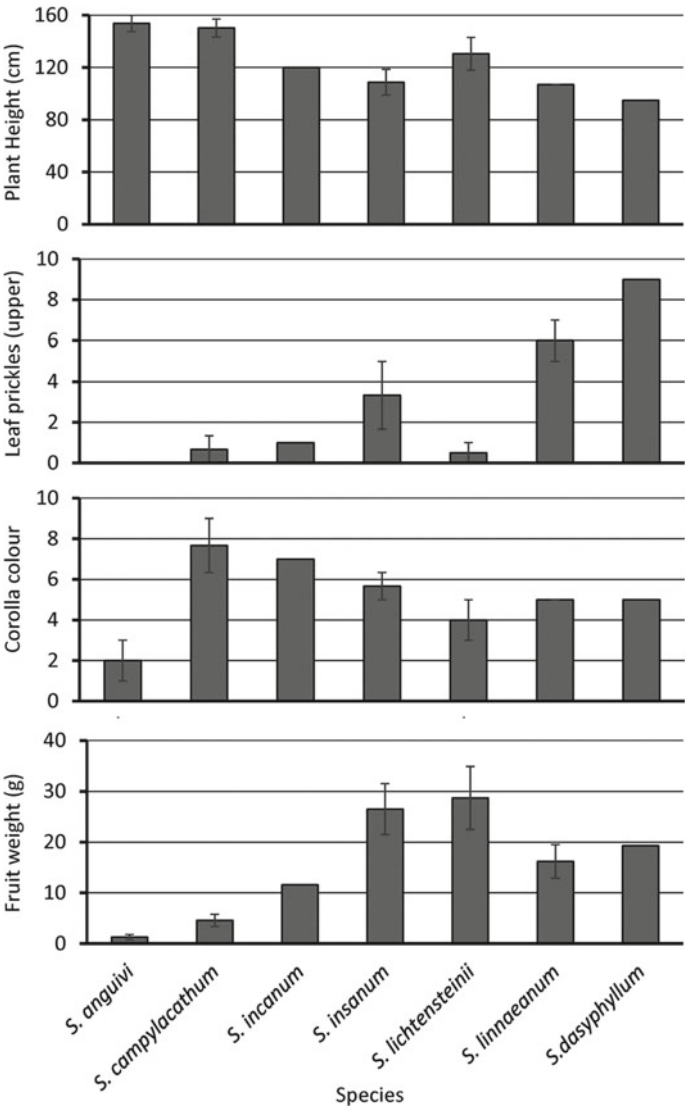

Fig. 4.2 Average (± SE) values for potentially adaptive traits based on accession means for several wild relatives of cultivated eggplant. Bars without SE are based on a single accession. Data taken from Kaushik et al. (2016) under the terms of the Creative Commons Attribution License (CC BY)

4.5 Mutational/Transgenic Approaches to Develop Climate-Resilient Eggplant

4.5.1 Mutagenic Approaches

A range of approaches have been applied to eggplant to induce mutations. Several studies have demonstrated morphological mutants, and in some cases these have led to the genetic characterization of pathways involved in these traits.

Of relevance to this article are fruit color mutants, which potentially have altered nutritional benefits (Xiao et al. 2016, 2017a). Xiao et al. have identified white, green, and black-purple fruits in a mutagenized population of the purple-fruited eggplant and demonstrated their anthocyanin contents differed. In addition, a number of dwarfing mutants were identified (Xiao et al. 2017a), and these could be resilient to lower water input, although was not tested, and may depend on the pathway which has been affected by the mutation (Lafitte et al. 2006). In another study, the total number of fruit was reduced in mutagenized eggplant populations, although the mass and size of fruits were generally increased (Prakash and Kumar 2018).

Improving eggplant biotic and abiotic tolerances through mutagenesis, however, is untested currently.

4.5.2 Transgenic Approaches

Eggplant is relatively amenable to *Agrobacterium*-mediated transformation, and this has been used since the late 1980s. Most studies have utilized information from other crops to identify suitable target genes for transformation, and have, in general, focused on pest and pathogen resistance. Early studies demonstrated that mutated forms of the *Bt* toxin from *Bacillus*, when transformed into eggplant, provided resistance to Colorado potato beetle, a major European and North American eggplant pest (Arpaia et al. 1997) and shoot and fruit borer (Kumar et al. 1998). In another study, resistance to the root-knot nematode *Meloidogyne* was conferred by the transformation of eggplant with the rice cystatin locus (Papolu et al. 2016).

In terms of resistance to abiotic stresses, one of the earliest successes in eggplant was the improved drought, chilling, and salinity tolerance in eggplants transformation with bacterial *mannitol-1-phosphodehydrogenase* (*mtlD*; Prabhavathi et al. 2002; Table 4.1). This study highlights the observation, which has been made in other crops too (reviewed in Golldack et al. 2014), that the genetic basis of different stresses maybe be identical, similar, or at least share some of the genetic components; thus, development of germplasm resistant to one stress may be in addition tolerant of other stresses. What was probably less expected was that the mannitol-producing eggplants also exhibited increased tolerance to a range of fungal wilts (Prabhavathi and Rajam 2007).

Table 4.1 Fresh weight (mean ± SE) for eggplant *mtlD* T1 transgenic seedlings (M) and untransformed controls grown in test tubes containing vermiculite: soil mix (1:1) and one-tenth MS liquid medium for 1 month with 200 mM NaCl (salt stress) and 10% PEG (drought)

	Salt		Drought	
	Seedling height (cm)	Fresh weight (mg)	Seedling height (cm)	Fresh weight (mg)
Control (with stress)	7.33 ± 0.33	0.026 ± 0.003	7.73 ± 0.37	0.03 ± 0.005
Control (no stress)	10.00 ± 0.00*	0.20 ± 0.003*	11.00 ± 0.58*	0.16 ± 0.005*
M1	9.66 ± 0.33*	0.24 ± 0.03*	7.13 ± 0.09	0.14 ± 0.005*
M8	8.66 ± 0.07*	0.21 ± 0.005*	6.16 ± 0.72	0.09 ± 0.005*
M9	9.21 ± 0.08*	0.18 ± 0.02*	8.77 ± 2.51	0.17 ± 0.01*
M16	9.71 ± 0.23*	0.16 ± 0.005*	7.71 ± 0.33	0.06 ± 0.01*

*indicates significant difference from control (with stress) at 5% level. Modified from Prabhavathi et al. (2002) with permission from Springer-Nature

Other studies have shown that a range of foreign genes can be transformed into eggplant to increase their tolerance to abiotic stresses. Transfer of *isopentytransperase* (*IPT*) under control of a senescent-specific promoter delayed senescence and increased tolerance to drought and chilling (Xiao et al. 2017b). Transgenic introduction of a wheat Na +/H+ antiporter encoded by the *TaNHX2* gene into eggplant increased tolerance of saline conditions (Yarra and Kirti 2019).

The public perception of transgenic technologies, the widespread ban on transgenic foods, and the extensive assessments on nontarget organisms required, probably limits the study of eggplants transgenics to identifying candidate genes and for scientific curiosity, and may explain why relatively little research is currently being undertaken, and why alternative approaches (specifically introgression from CWRs; see above) are more common.

4.6 Other Eggplants

The scarlet eggplant (*S. aethiopicum* L.) and the Gboma eggplant (*S. macrocarpon* L.) are grown for human consumption; however, not to the same extent as *S. melongena*. The leaves of both species are also consumed. Both are in the secondary gene pool of *S. melongena*, and results from crosses between the cultivated eggplants are extremely variable (reviewed in Daunay et al. 2019). Crosses between *S. aethiopicum* and the other two species generally result in a vigorous F_1 with fertility from sterile to partially fertile, whereas the cross between *S. macrocarpon* and *S. melongena*, the F_1,

is generally weak. However, in all cases, the F_1 have set seed, and/or later generation crosses have been obtained (reviewed in Daunay et al. 2019).

Phenotyping of *S. aethiopicum* and *S. macrocarpon* has been carried out on smaller number of accessions than phenotyping of the Asian eggplant, however, has revealed some important characters that distinguish these species (Plazas et al. 2014; San José et al. 2016). In addition, it was found that the two African eggplants had greater fiber and vitamin C content than Asian eggplant, and that *S. macrocarpon* contains more phenolics (powerful antioxidants) than *S. aethiopicum* (San José et al. 2016). The African eggplants are differently adapted to *S. melongena* and could provide some interesting focal species with respect to resistance to warmer climate and varying precipitation.

Overall it seems that these African eggplants contain certain nutritional benefits over *S. melongena*; however, ensuring that these properties are maintained under climate change-relevant environments has rarely been assessed. However, in one study, the content of various carotenoids in multiple accessions of the two African eggplants was assessed in control and drought-affected plants (Mibei et al. 2017). The study revealed that carotenes, chlorophylls, neoxanthin, and violaxanthin decreased under water stress, however zeaxanthin content increased under stress and lutein was unaffected. This valuable insight tells us that climate change could affect the nutrient content of these eggplants, and more attention should be paid to the effect of the changing climate on nutritional compounds.

Solanum aethiopicum and *S. macrocarpon* exhibit good resistance to *Fusarium* (Daunay et al. 1991) and the former has been crossed with Asian eggplant via protoplast fusion and callus regeneration to make segregating populations (Toppino et al. 2008). The analysis identified a single locus controlling resistance to *Fusarium* and PCR (polymerase chain reaction)-based markers were developed to allow the expedited breeding of further backcross progenies.

Interestingly, the potential for *S. melongena* to improve the African eggplants has not been studied, but hypothetically, introgression of *S. melongena* alleles into *S. macrocarpon* could be used to increase variation in fruit shape, which is currently rather invariant (Page et al. 2019a).

4.7 Future Perspectives

From this review, it appears there are many research avenues being explored which assess the ability of eggplant to cope with future climates, and to identify adaptive germplasm. However, the research also appears to be progressing at a relatively slow pace, compared to other crops, which poses a risk to the development of climate change-resilient eggplant which could be needed in just the next few decades. Until recently, the eggplant genome available (Hirakawa et al. 2014) was quite highly fragmented, whereas it is anticipated that a significantly better assembly will be made available soon (Gramazio et al. 2018).

I highlight here three research avenues which should be prioritized for the enhancement of eggplant tolerance to future climates.

4.7.1 Development of Introgression Lines

The development of introgression lines (ILs; crop varieties with introgressed genome segments from a related species), has the potential to help gain an understanding of the genetic basis of adaptive traits, and serve as pre-breeding material. These are only just being developed in eggplant (Kouassi et al. 2016), and are only well-developed for one IL population (Gramazio et al. 2017). In contrast, IL populations are extensively used in understanding adaptive phenotypes in tomato (e.g., Eshed et al. 1996; Fridman et al. 2000) and were developed over 25 years ago (Eshed et al. 1992).

The development of these lines is relatively time-consuming, but once developed they can be "immortalized" as populations that the research community can share. This means multiple investigations into adaptive traits can take place in the same germplasm. Because the presence of genome sequences greatly aids in the identification of genetic variants underlying said traits, the parents of the ILs can be sequenced and then used as a resource by multiple groups, reducing the need for different research groups to obtain genome sequences of multiple IL populations.

Early steps have been taken to develop ILs in a range of wild x cultivated eggplant crosses (Kouassi et al. 2016) and it is hoped that these can be developed further. Utilizing genetic markers and targeting specific introgressions, it can be relatively quick to recover cultivar-like plants containing the wild-like trait of interest (Tanksley and McCouch 1997).

4.7.2 Conservation and Study of Eggplant Crop-Wild Relatives

Future climates will require a range of adaptations not present in the eggplant gene pool and introgression from the wild is a potential source of these adaptations. As made evident earlier in this chapter, eggplant crop-wild relatives (CWRs) host a range of important adaptations that could be utilized in breeding eggplant for future climates. This ranges from drought (e.g., *S. lichtensteinii* and *S. incanum*) and salinity tolerance (*S. linnaeanum*) to the tolerance of a range of pathogens (*S. torvum*and *S. linnaeanum*). In some cases, adaptive traits from CWRs have been introgressed into eggplant (Liu et al. 2015; Rotino et al. 2014), although these studies are not common. Further studies of wild species are needed; however, it is also important that a range of germplasm from the more widespread species which inhabit diverse environments

(e.g., *S. campylacanthum* and *S. insanum*) are investigated, instead of relying on one or a few accessions.

This may be difficult currently because a number of eggplant CWRs are poorly represented in gene banks (Taher et al. 2017). In a systematic survey of eggplant CWRs, cross-referenced with gene banks, it was recently shown that several eggplant CWRs should be considered high priority for future collection, and this included a number of species previously mentioned in this review which are known for their biotic and abiotic tolerances (Syfert et al. 2016). In addition, 14 eggplant CWRs are threatened or near-threatened in the wild (Syfert et al. 2016). Given that, in other crop-CWR groups, climate change is estimated to have a significant negative impact (Jarvis et al. 2008), it is important we identify and conserve eggplant CWRs now.

4.7.3 Functional Analysis of Adaptive Traits

While ILs and large mapping populations aid in the understanding of QTLs underlying traits of interest, gaining knowledge of the specific genes controlling these traits would provide several advantages.

First, identifying the gene controlling an adaptation is useful in applying MAS to breeding material. In MAS, large numbers of crosses can be rapidly screened for the presence of molecular markers flanking QTLs of interest, and those crosses not containing the markers (and therefore the QTL) removed early on, expediting the process.

Second, we can screen for novel variation across germplasm (both eggplant and the CWRs) and assess if the same or different genes control the trait of interest. If different genes control the same trait in different germplasms, then there is the potential to further increase tolerance or resistance by introgressing from multiple sources.

Third, to employ modern gene editing technologies, one needs to understand the genetic basis of the trait being investigated. CRISPR/Cas9, a widely employed gene editing technology (Jinek et al. 2012), requires guide RNAs to be designed which complements the locus of interest, upon which the Cas9 endonuclease makes a targeted lesion.

Understanding the genetic basis of certain traits in eggplant is being carried out, but is generally in its infancy, especially when comparing to other crops. Successful identification of genes controlling resistance to Fusarium wilt has been carried out (Mutlu et al. 2008; Boyaci et al. 2012), and functional characterization of bacterial wilt resistance is a current focus of study (Xiao et al. 2015; Morel et al. 2018).

4.8 Conclusions

In order to prepare for a warmer climate, more prone to droughts and floods, and with the potential for novel pests and pathogens to become a threat, it is vital that current research identifies crop varieties and CWRs with adaptive tolerance to these stresses. While this is being carried out for eggplant, it appears that progress is slow and, until very recently, has lacked behind other crops. While eggplant is not globally one of the most important vegetables, it plays a significant part in the diet of many countries and cultures, and any loss of production could harm these populations. In this review, I have highlighted what we already know about eggplant tolerances that may be of use in a future climate, and also highlight some important research avenues which should be prioritized.

References

Achuo EA, Prinsen E, Höfte M (2006) Influence of drought, salt stress and abscisic acid on the resistance of tomato to *Botrytis cinerea* and *Oidium neolycopersici*. Plant Pathol 55(2):178–186. https://doi.org/10.1111/j.1365-3059.2006.01340.x

Ahuja I, de Vos RC, Bones AM, Hall RD (2010) Plant molecular stress responses face climate change. Trends Plant Sci 15(12):664–674. https://doi.org/10.1016/j.tplants.2010.08.002

Arpaia S, Mennella G, Onofaro V, Perri E, Sunseri F, Rotino GL (1997) Production of transgenic eggplant (*Solanum melongena* L.) resistant to Colorado Potato Beetle (*Leptinotarsa decemlineata* Say). Theor Appl Genet 95 (3):329–334. https://doi.org/10.1007/s001220050567

Atlin GN, Cairns JE, Das B (2017) Rapid breeding and varietal replacement are critical to adaptation of cropping systems in the developing world to climate change. Glob Food Secur 12:31–37. https://doi.org/10.1016/j.gfs.2017.01.008

Aubriot X, Knapp S, Syfert MM, Poczai P, Buerki S (2018) Shedding new light on the origin and spread of the brinjal eggplant (*Solanum melongena* L.) and its relatives. Amer J Bot 105 (7):1175–1187. https://doi.org/10.1002/ajb2.1133

Bahadur A, Rai N, Kumar R, Tiwari SK, Singh AK, Rai AK, Singh U, Patel PK, Tiwari V, Rai AB, Singh M, Singh BB (2015) Grafting tomato on eggplant as a potential tool to improve waterlogging tolerance in hybrid tomato. Veg Sci 42:82–87

Bletsos FA, Roupakias DG, Tsaktsira ML, Scaltsoyjannes AB, Thanassoulopoulos CC (1998) Interspecific hybrids between three eggplant (*Solanum melongena* L.) cultivars and two wild species (*Solanum torvum* Sw. and *Solanum sisymbriifolium* Lam.). Plant Breed 117 (2):159–164. https://doi.org/10.1111/j.1439-0523.1998.tb01471.x

Boyaci F, Unlu A, Abak K (2012) Screening for resistance to *Fusarium* wilt of some cultivated eggplants and wild *Solanum* accessions. In: Leitao JM (ed) XXVIII International horticultural congress on science and horticulture for people, vol 935. Acta Horticulturae. Intl Soc Horticultural Science, Lisbon (Portugal), pp 23–27

Bui HH, Serra V, Pagès L (2015) Root system development and architecture in various genotypes of the Solanaceae family. Botany 93(8):465–474. https://doi.org/10.1139/cjb-2015-0008

Challinor AJ, Koehler AK, Ramirez-Villegas J, Whitfield S, Das B (2016) Current warming will reduce yields unless maize breeding and seed systems adapt immediately. Nat Clim Change 6:954. https://doi.org/10.1038/nclimate3061

Christodoulakis NS, Lampri P-N, Fasseas C (2009) Structural and cytochemical investigation of the leaf of silverleaf nightshade (*Solanum elaeagnifolium*), a drought-resistant alien weed of the Greek flora. Aust J Bot 57(5):432–438. https://doi.org/10.1071/BT08210

Collonnier U, Fock I, Daunay MC, Servaes A, Vedel F, Sijak-Yakovlev S, Souvannavong V, Sihachakr D (2003) Somatic hybrids between *Solanum melongena* and *S. sisymbriifolium*, as a useful source of resistance against bacterial and fungal wilts. Plant Sci 164 (5):849–861. https://doi.org/10.1016/s0168-9452(03)00075-x

Coumou D, Rahmstorf S (2012) A decade of weather extremes. Nat Clim Change 2:491. https://doi.org/10.1038/nclimate1452

Daunay M-C, Salinier J, Aubriot X (2019) Crossability and diversity of eggplants and their wild relatives. In: Chapman MA (ed) The eggplant genome. Springer, Cham, Switzerland, pp 135–191

Daunay MC (2008) Eggplant. In: Prohens J, Nuez F (eds) Vegetables II: Fabaceae, Liliaceae, Solanaceae, and Umbelliferae. Springer, New York, NY, USA, pp 163–220

Daunay MC, Lester RN, Laterrot H (1991) The use of wild species for the genetic improvement of brinjal eggplant (*Solanum melongena*) and tomato (*Lycopersicum esculentum*). In: Hawkes JG, Lester RN, Nee M, Estrada N (eds) Solanaceae III: Taxonomy, chemistry, and evolution. Royal Botanic Gardens, Kew, UK, Royal Botanic Gardens, Kew

Davidar P, Snow AA, Rajkumar M, Pasquet R, Daunay MC, Mutegi E (2015) The potential for crop to wild hybridization in eggplant (*Solanum melongena*; Solanaceae) in southern India. Amer J Bot 102(1):129–139. https://doi.org/10.3732/ajb.1400404

Dempewolf H, Eastwood RJ, Guarino L, Khoury CK, Müller JV, Toll J (2014) Adapting agriculture to climate change: a global initiative to collect, conserve, and use crop wild relatives. Agroecol Sustain Food Syst 38(4):369–377. https://doi.org/10.1080/21683565.2013.870629

Doganlar S, Frary A, Daunay MC, Lester RN, Tanksley SD (2002a) A comparative genetic linkage map of eggplant (*Solanum melongena*) and its implications for genome evolution in the Solanaceae. Genetics 161(4):1697–1711

Doganlar S, Frary A, Daunay MC, Lester RN, Tanksley SD (2002b) Conservation of gene function in the Solanaceae as revealed by comparative mapping of domestication traits in eggplant. Genetics 161(4):1713–1726

Donatelli M, Magarey RD, Bregaglio S, Willocquet L, Whish JPM, Savary S (2017) Modelling the impacts of pests and diseases on agricultural systems. Agri Syst 155:213–224. https://doi.org/10.1016/j.agsy.2017.01.019

Eshed Y, Abu-Abied M, Saranga Y, Zamir D (1992) *Lycopersicon esculentum* lines containing small overlapping introgressions from *L. pennellii*. Theor Appl Genet 83 (8):1027–1034

Eshed Y, Gera G, Zamir D (1996) A genome-wide search for wild-species alleles that increase horticultural yield of processing tomatoes. Theor Appl Genet 93(5–6):877–886. https://doi.org/10.1007/bf00224089

FAO (2017) FAOSTAT database collections (http://faostat.fao.org/). Food and Agriculture Organization of the United Nations, Rome, Italy. September 2017

Fita A, Fioruci F, Plazas M, Rodriguez-Burruezo A, Prohens J (2015) Drought tolerance among accessions of eggplant and related species. Bull UASVM Hort 72:461–462

Fridman E, Pleban T, Zamir D (2000) A recombination hotspot delimits a wild-species quantitative trait locus for tomato sugar content to 484 bp within an invertase gene. Proc Natl Acad Sci USA 97:4718–4723

Garcia-Fortea E, Gramazio P, Vilanova S, Fita A, Mangino G, Villanueva G, Arrones A, Knapp S, Prohens J, Plazas M (2019) First successful backcrossing towards eggplant (*Solanum melongena* L.) of a new world species, the silverleaf nightshade (*S. elaeagnifolium*), and characterization of interspecific hybrids and backcrosses. Sci Hort 246:563–573

García-Salas P, Gómez-Caravaca AM, Morales-Soto A, Segura-Carretero A, Fernández-Gutiérrez A (2014) Identification and quantification of phenolic compounds in diverse cultivars of eggplant grown in different seasons by high-performance liquid chromatography coupled to diode array detector and electrospray-quadrupole-time of flight-mass spectrometry. Food Res Int 57:114–122. https://doi.org/10.1016/j.foodres.2014.01.032

Garrett KA, Dendy SP, Frank EE, Rouse MN, Travers SE (2006) Climate change effects on plant disease: genomes to ecosystems. Annu Rev Phytopathol 44:489–509. https://doi.org/10.1146/annurev.phyto.44.070505.143420

Gilliham M, Able JA, Roy SJ (2017) Translating knowledge about abiotic stress tolerance to breeding programmes. Plant J 90(5):898–917. https://doi.org/10.1111/tpj.13456
Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM, Toulmin C (2010) Food security: the challenge of feeding 9 billion people. Science 327(5967):812–818. https://doi.org/10.1126/science.1185383
Golldack D, Li C, Mohan H, Probst N (2014) Tolerance to drought and salt stress in plants: unraveling the signaling networks. Front Plant Sci 5:151–151. https://doi.org/10.3389/fpls.2014.00151
Gramazio P, Prohens J, Plazas M, Mangino G, Herraiz FJ, Garcia-Fortea E, Vilanova S (2018) Genomic tools for the enhancement of vegetable crops: a case in eggplant. Not Bot Horti Agrobot Cluj-Napoca 46(1):1–13. https://doi.org/10.15835/nbha46110936
Gramazio P, Prohens J, Plazas M, Mangino G, Herraiz FJ, Vilanova S (2017) Development and genetic characterization of advanced backcross materials and an introgression line population of *Solanum incanum* in a *S. melongena* background. Front Plant Sci 8 (1477). https://doi.org/10.3389/fpls.2017.01477
Hajjar R, Hodgkin T (2007) The use of wild relatives in crop improvement: a survey of developments over the last 20 years. Euphytica 156(1):1–13. https://doi.org/10.1007/s10681-007-9363-0
Harlan JR, de Wet JMJ (1971) Toward a rational classification of cultivated plants. Taxon 20:509–517
Hirakawa H, Shirasawa K, Miyatake K, Nunome T, Negoro S, Ohyama A, Yamaguchi H, Sato S, Isobe S, Tabata S, Fukuoka H (2014) Draft genome sequence of eggplant (*Solanum melongena* L.): the representative *Solanum* species indigenous to the old world. DNA Res 21 (6):649–660. https://doi.org/10.1093/dnares/dsu027
Jarl CI, Rietveld EM, de Haas JM (1999) Transfer of fungal tolerance through interspecific somatic hybridisation between *Solanum melongena* and *S. torvum*. Plant Cell Rep 18 (9):791–796. https://doi.org/10.1007/s002990050663
Jarvis A, Lane A, Hijmans RJ (2008) The effect of climate change on crop wild relatives. Agri Ecosyst Environ 126(1–2):13–23. https://doi.org/10.1016/j.agee2008.01.013
Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E (2012) A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. Science 337(6096):816–821. https://doi.org/10.1126/science.1225829
Kashyap V, Kumar SV, Collonnier C, Fusari F, Haicour R, Rotino GL, Sihachakr D, Rajam M (2003) Biotechnology of eggplant. Sci Hort 97(1):1–25. https://doi.org/10.1016/s0304-4238(02)00140-1
Kaushik P, Prohens J, Vilanova S, Gramazio P, Plazas M (2016) Phenotyping of eggplant wild relatives and interspecific hybrids with conventional and phenomics descriptors provides insight for their potential utilization in breeding. Front Plant Sci 7:677. https://doi.org/10.3389/fpls.2016.00677
Knapp S, Vorontsova MS, Prohens J (2013) Wild relatives of the eggplant (*Solanum melongena* L.: Solanaceae): new understanding of species names in a complex group. PLoS One8 (2):e57039. https://doi.org/10.1371/journal.pone.0057039
Kouassi B, Prohens J, Gramazio P, Kouassi AB, Vilanova S, Galan-Avila A, Herraiz FJ, Kouassi A, Segui-Simarro JM, Plazas M (2016) Development of backcross generations and new interspecific hybrid combinations for introgression breeding in eggplant (*Solanum melongena*). Sci Hort 213:199–207. https://doi.org/10.1016/j.scienta.2016.10.039
Kumar PA, Mandaokar A, Sreenivasu K, Chakrabarti SK, Bisaria S, Sharma SR, Kaur S, Sharma RP (1998) Insect-resistant transgenic brinjal plants. Mol Breed 4(1):33–37. https://doi.org/10.1023/a:1009694016179
Kumchai J, Wei YC, Lee CY, Chen FC, Chin SW (2013) Production of interspecific hybrids between commercial cultivars of the eggplant (*Solanum melongena* L.) and its wild relative *S. torvum*. Gen Mol Res 12 (1):755–764. https://doi.org/10.4238/2013.March.13.4
Lafitte H, Li Z-K, Yongsheng G, Yan S (2006) Whole plant responses, key processes, and adaptation to drought stress: the case of rice. J Exp Bot 58(2):169–175. https://doi.org/10.1093/jxb/erl101
Lebeau A, Daunay MC, Frary A, Palloix A, Wang JF, Dintinger J, Chiroleu F, Wicker E, Prior P (2011) Bacterial wilt resistance in tomato, pepper, and eggplant: genetic resources respond to

diverse strains in the *Ralstonia solanacearum* species complex. Phytopathology 101(1):154–165. https://doi.org/10.1094/phyto-02-10-0048

Lebeau A, Gouy M, Daunay MC, Wicker E, Chiroleu F, Prior P, Frary A, Dintinger J (2013) Genetic mapping of a major dominant gene for resistance to Ralstonia solanacearum in eggplant. Theor Appl Genet 126(1):143–158. https://doi.org/10.1007/s00122-012-1969-5

Lee J-M, Kubota C, Tsao SJ, Bie Z, Echevarria PH, Morra L, Oda M (2010) Current status of vegetable grafting: diffusion, grafting techniques, automation. Sci Hort 127(2):93–105. https://doi.org/10.1016/j.scienta.2010.08.003

Lester RN, Hasan SMZ (1991) Origin and domestication of the brinjal eggplant, *Solanum melongena*, from *S. incanum* in Africa and Asia. In: Hawkes JG, Lester RN, Nee M, Estrada N (eds) Solanaceae III: taxonomy, chemistry, evolution. UK, Royal Botanic Gardens, Kew, pp 369–387

Lester RN, Kang JH (1998) Embryo and endosperm function and failure in *Solanum* species and hybrids. Ann Bot 82(4):445–453. https://doi.org/10.1006/anbo.1998.0695

Liu J, Zheng Z, Zhou X, Feng C, Zhuang Y (2015) Improving the resistance of eggplant (*Solanum melongena*) to verticillium wilt using wild species *Solanum linnaeanum*. Euphytica 201(3):463–469. https://doi.org/10.1007/s10681-014-1234-x

Maxted N, Ford-Lloyd BV, Kell SP, Iriondo J, Dulloo E, Turok J (2007) Crop wild relative conservation and use. CAB International, Wallingford, UK

Maxted N, Kell SP (2009) Establishment of a global network for the in situ conservation of crop wild relatives: status and needs. FAO Consultancy Report, FAO, Rome

Mibei EK, Ambuko J, Giovannoni JJ, Onyango AN, Owino WO (2017) Carotenoid profiling of the leaves of selected African eggplant accessions subjected to drought stress. Food Sci Nutr 5(1):113–122. https://doi.org/10.1002/fsn3.370

Minghua Y, Yuejin X, Xiaoli L, Li Y (2001) Studies on biochemical and physiological indices of chilling tolerance in eggplant. Acta Hort Sin 19:1

Mittler R (2006) Abiotic stress, the field environment and stress combination. Trends Plant Sci 11(1):15–19. https://doi.org/10.1016/j.tplants.2005.11.002

Miyatake K, Saito T, Negoro S, Yamaguchi H, Nunome T, Ohyama A, Fukuoka H (2016) Detailed mapping of a resistance locus against *Fusarium* wilt in cultivated eggplant (*Solanum melongena*). Theor Appl Genet 129(2):357–367. https://doi.org/10.1007/s00122-015-2632-8

Morel A, Guinard J, Lonjon F, Sujeeun L, Barberis P, Genin S, Vailleau F, Daunay MC, Dintinger J, Poussier S, Peeters N, Wicker E (2018) The eggplant AG91-25 recognizes the Type III-secreted effector RipAX2 to trigger resistance to bacterial wilt (*Ralstonia solanacearum* species complex). Mol Plant Pathol 19(11):2459–2472. https://doi.org/10.1111/mpp.12724

Morrell PL, Buckler ES, Ross-Ibarra J (2011) Crop genomics: advances and applications. Nat Rev Genet 13:85. https://doi.org/10.1038/nrg3097

Mutlu N, Boyaci FH, Gocmen M, Abak K (2008) Development of SRAP, SRAP-RGA, RAPD and SCAR markers linked with a Fusarium wilt resistance gene in eggplant. Theor Appl Genet 117(8):1303–1312. https://doi.org/10.1007/s00122-008-0864-6

Oerke E-C, Dehne H-W, Schonbeck F, Weber A (1994) Crop production and crop protection: estimated losses in major food and cash crops. Elsevier Science, Amsterdam

Page AML, Daunay M-C, Aubriot X, Chapman MA (2019a) Domestication of eggplants: a phenotypic and genomic insight. In: Chapman MA (ed) The eggplant genome. Springer, Cham, Switzerland, pp 193–212

Page AML, Gibson J, Meyer RS, Chapman MA (2019b) Eggplant domestication: pervasive gene flow, feralisation and transcriptomic divergence. Mol Biol Evol 36 (7): 1359–1372. https://doi.org/10.1093/molbev/msz062

Papolu PK, Dutta TK, Tyagi N, Urwin PE, Lilley CJ, Rao U (2016) Expression of a cystatin transgene in eggplant provides resistance to root-knot nematode, *Meloidogyne incognita*. Front Plant Sci 7:1122. https://doi.org/10.3389/fpls.2016.01122

Plazas M, Andujar I, Vilanova S, Gramazio P, Herraiz FJ, Prohens J (2014) Conventional and phenomics characterization provides insight into the diversity and relationships of hypervariable

scarlet (*Solanum aethiopicum* L.) and gboma (*S. macrocarpon* L.) eggplant complexes. Front Plant Sci 5: 318. https://doi.org/10.3389/fpls.2014.00318

Plazas M, Vilanova S, Gramazio P, Rodriguez-Burruezo A, Fita A, Herraiz FJ, Ranil R, Fonseka R, Niran L, Fonseka H, Kouassi B, Kouassi A, Prohens J (2016) Interspecific hybridization between eggplant and wild relatives from different genepools. J Amer Soc Hort Sci 141(1):34–44

Poppy GM, Chiotha S, Eigenbrod F, Harvey CA, Honzak M, Hudson MD, Jarvis A, Madise NJ, Schreckenberg K, Shackleton CM, Villa F, Dawson TP (2014) Food security in a perfect storm: using the ecosystem services framework to increase understanding. Phil Trans Ro Soc B-Biol Sci 369. doi:10.1098/rstb.2012.0288

Prabhavathi V, Rajam MV (2007) Mannitol-accumulating transgenic eggplants exhibit enhanced resistance to fungal wilts. Plant Sci 173(1):50–54. https://doi.org/10.1016/j.plantsci.2007.04.004

Prabhavathi V, Yadav JS, Kumar PA, Rajam MV (2002) Abiotic stress tolerance in transgenic eggplant (*Solanum melongena* L.) by introduction of bacterial mannitol phosphodehydrogenase gene. Mol Breed 9 (2):137–147. https://doi.org/10.1023/a:1026765026493

Prakash J, Kumar S (2018) Effect of mutagens on induced variability in eggplant (*Solanum melongena*). Indian J Agri Sci 83:1269–1279

Ranil RHG, Prohens J, Aubriot X, Niran HML, Plazas M, Fonseka RM, Vilanova S, Fonseka HH, Gramazio P, Knapp S (2017) *Solanum insanum* L. (subgenus *Leptostemonum* Bitter, Solanaceae), the neglected wild progenitor of eggplant (*S. melongena* L.): a review of taxonomy, characteristics and uses aimed at its enhancement for improved eggplant breeding. Genet Resour Crop Evol 64 (7):1707–1722. https://doi.org/10.1007/s10722-016-0467-z

Rao GR, Kumar A (1980) Some observations on interspecific hybrids of *Solanum melongena* L. Proceedings: Plant Sci 89 (2):117–121. https://doi.org/10.1007/bf03046156

Reusche M, Thole K, Janz D, Truskina J, Rindfleisch S, Drubert C, Polle A, Lipka V, Teichmann T (2012) *Verticillium* infection triggers VASCULAR-RELATED NAC DOMAIN7-dependent de novo xylem formation and enhances drought tolerance in *Arabidopsis*. Plant Cell 24(9):3823–3837. https://doi.org/10.1105/tpc.112.103374

Rotino GL, Sala T, Toppino L (2014) Eggplant. In: Pratap A, Kumar J (eds) Alien gene transfer in crop plants. Springer, NewYork, pp 381–409

Rouhani I, Black CC Jr, Vines HM, Kormanik PP (1987) Effect of number of lateral roots on eggplant growth and yield. Can J Plant Sci 67(1):305–313. https://doi.org/10.4141/cjps87-044

Sadashiva AT, Reddy KM, Ganeshan G, Prasad BCN (2001) Evaluation of eggplant (*Solanum melongena* L.) lines for bacterial wilt resistance. Capsicum Eggplant Newslr 20:117–119

Salgon S, Raynal M, Lebon S, Baptiste JM, Daunay MC, Dintinger J, Jourda C (2018) Genotyping by sequencing highlights a polygenic pesistance to *Ralstonia pseudosolanacearum* in eggplant (*Solanum melongena* L.). Intl JMol Sci 19(2). https://doi.org/10.3390/ijms19020357

San José R, Plazas M, Sánchez-Mata MC, Cámara M, Prohens J (2016) Diversity in composition of scarlet (*S. aethiopicum*) and gboma (*S. macrocarpon*) eggplants and of interspecific hybrids between *S. aethiopicum* and common eggplant (*S. melongena*). J Food Compos Anal 45:130–140. https://doi.org/10.1016/j.jfca.2015.10.009

Savary S, Ficke A, Aubertot J-N, Hollier C (2012) Crop losses due to diseases and their implications for global food production losses and food security. Food Secur 4(4):519–537. https://doi.org/10.1007/s12571-012-0200-5

Schippers RR (2002) African indigenous vegetables: an overview of the cultivated species.University of Greenwich, Natural Resources Institute, pp 221

Sunseri F, Sciancalepore A, Martelli G, Acciarri N, Rotino GL, Valentino D, Tamietti G (2003) Development of RAPD-AFLP map of eggplant and improvement of tolerance to verticillium wilt. Acta Hort 625:107–115. https://doi.org/10.17660/ActaHortic.2003.625.10

Syfert MM, Castaneda-Alvarez NP, Khoury CK, Sarkinen T, Sosa CC, Achicanoy HA, Bernau V, Prohens J, Daunay MC, Knapp S (2016) Crop wild relatives of the brinjal eggplant (*Solanum melongena*): poorly represented in genebanks and many species at risk of extinction. Amer J Bot 103(4):635–651. https://doi.org/10.3732/ajb.1500539

Taher D, Solberg SO, Prohens J, Chou YY, Rakha M, Wu TH (2017) World Vegetable Center eggplant collection: origin, composition, seed dissemination and utilizationin breeding. Front Plant Sci 8. doi:10.3389/fpls.2017.01484

Tani E, Kizis D, Markellou E, Papadakis I, Tsamadia D, Leventis G, Makrogianni D, Karapanos I (2018) Cultivar-dependent responses of eggplant (*Solanum melongena* L.) to simultaneous *Verticillium dahliae* infection and drought. Front Plant Sci 9:1181–1181. https://doi.org/10.3389/fpls.2018.01181

Tanksley SD, McCouch SR (1997) Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277(5329):1063–1066

Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S (2002) Agricultural sustainability and intensive production practices. Nature 418(6898):671–677

Toppino L, Barchi L, Lo Scalzo R, Palazzolo E, Francese G, Fibiani M, D'Alessandro A, Papa V, Laudicina VA, Sabatino L, Pulcini L, Sala T, Acciarri N, Portis E, Lanteri S, Mennella G, Rotino GL (2016) Mapping quantitative trait loci affecting biochemical and morphological fruit properties in eggplant (*Solanum melongena* L.). Front Plant Sci 7:256. https://doi.org/10.3389/fpls.2016.00256

Toppino L, Valè G, Rotino GL (2008) Inheritance of Fusarium wilt resistance introgressed from *Solanum aethiopicum* Gilo and Aculeatum groups into cultivated eggplant (*S. melongena*) and development of associated PCR-based markers. Mol Breed 22 (2):237–250. https://doi.org/10.1007/s11032-008-9170-x

Trenberth KE, Dai A, van der Schrier G, Jones PD, Barichivich J, Briffa KR, Sheffield J (2013) Global warming and changes in drought. Nat Clim Change 4:17. https://doi.org/10.1038/nclimate2067

Vorontsova MS, Knapp S (2012) *Solanum* section *Melongena*. In: Beentje H (ed) Flora of tropical East Africa. Royal Botanic Gardens, Kew, UK, pp 198–215

Vorontsova MS, Stern S, Bohs L, Knapp S (2013) African spiny *Solanum* (subgenus *Leptostemonum*, Solanaceae): a thorny phylogenetic tangle. Bot J Linn Soc 173(2):176–193. https://doi.org/10.1111/boj.12053

Warschefsky E, Penmetsa RV, Cook DR, von Wettberg EJ (2014) Back to the wilds: tapping evolutionary adaptations for resilient crops through systematic hybridization with crop wild relatives. Amer J Bot 101(10):1791–1800. https://doi.org/10.3732/ajb.1400116

World Bank (2013) Turn down the heat: climate extremes, regional impacts, and the case for resilience. A report for the world bank by the Potsdam Institute for climate impact research and climate analytics. Washington, DC

Xiao XO, Cao BH, Li G, Lei J, Chen Q, Jiang J, Cheng YJ (2015) Functional characterization of a putative bacterial wilt resistance gene (RE-bw) in eggplant. Plant Mol Biol Rep 33(4):1058–1073. https://doi.org/10.1007/s11105-014-0814-1

Xiao XO, Lin W, Li W, Gao X, Lv L, Ma F, Liu Y (2017a) The analysis of physiological variations in M2 generation of *Solanum melongena* L. mutagenized by Ethyl Methane Sulfonate. Front Plant Sci 8 (17). https://doi.org/10.3389/fpls.2017.00017

Xiao XO, Lin WQ, Li W, Liu GQ, Zhang XH, Lv LL (2016) Creating new eggplant germplasm by EMS mutation. J S Agri 47:1247–1253

Xiao XO, Zeng YM, Cao BH, Lei JJ, Chen QH, Meng CM, Cheng YJ (2017b) PSAG12-IPT overexpression in eggplant delays leaf senescence and induces abiotic stress tolerance. J Hort Sci Biotechnol 92(4):349–357. https://doi.org/10.1080/14620316.2017.1287529

Yarra R, Kirti PB (2019) Expressing class I wheat *NHX* (*TaNHX2*) gene in eggplant (*Solanum melongena* L.) improves plant performance under saline condition. FunctiIntegr Genom https://doi.org/10.1007/s10142-019-00656-5

Yaşar F (2003) Investigation of some antioxidant enzyme activities in eggplant genotypes grown under salt stress in vitro and in vivo. PhD thesis, Institute of Natural and Applied Science, University of Yuzuncu Yıl. Turkey

Zhao C, Liu B, Piao S, Wang X, Lobell DB, Huang Y, Huang M, Yao Y, Bassu S, Ciais P, Durand J-L, Elliott J, Ewert F, Janssens IA, Li T, Lin E, Liu Q, Martre P, Müller C, Peng S, Peñuelas J, Ruane AC, Wallach D, Wang T, Wu D, Liu Z, Zhu Y, Zhu Z, Asseng S (2017) Temperature increase reduces global yields of major crops in four independent estimates. Proc Natl Acad Sci USA 114(35):9326–9331. https://doi.org/10.1073/pnas.1701762114

Chapter 5
Improving Vegetable Capsicums for Fruit Yield, Quality, and Tolerance to Biotic and Abiotic Stresses

Bala Rathinasabapathi

Abstract Sweet and pungent peppers (*Capsicum* spp.) are globally important vegetable and spice commodities as they are valued for their nutritional qualities, antioxidant compounds, flavors, pungency, brilliant colors, and textures. *Capsicum*. has extraordinary variability in its germplasm both in cultivated and wild species. This review presents an account of research done over several decades in the context of crop improvement. Key developments with reference to linkage analyses, DNA-based markers, the identification of quantitative trait loci for complex traits, transcriptomes of ripening fruit, and genome sequences are summarized. Prospects are excellent for using conventional, biotechnological, and genomic approaches to improve fruit yield, fruit quality, and biotic stress tolerance so that productivity in this specialty crop could be sustained, despite the changing climate. However, more research is needed to build resources to improve peppers for tolerance to abiotic stress factors.

Keywords Climate change · Disease resistance · Pepper · Pericarp quality · Root-knot nematode resistance · Stress tolerance · Yield

5.1 Capsicum: Production, Uses, and Breeding Goals

Peppers (*Capsicum* spp.) are economically important crops, extensively used worldwide both for vegetable uses and for flavoring a variety of food items. Peppers contain a multitude of phytochemicals—pungent capsaicinoids, colorful carotenoids, antioxidant flavonoids, and flavor volatiles (Antonio et al. 2018). The phytochemical content and composition of peppers provide brilliant colors, nutritional value, and flavors to food unmatched by any other vegetable or spice. World production of fresh and dry peppers is about 36 and 4.6 million tons per year, in a total area of 1.9 and 1.85 million hectares, respectively (FAO 2017). Worldwide fresh pepper production has steadily increased about threefold since 1994 (FAO 2017). China is the top producer followed by Mexico, Turkey, Indonesia, Spain, USA, Nigeria, Egypt, Republic of Korea, and Italy.

B. Rathinasabapathi (✉)
Horticultural Sciences Department, University of Florida, Gainesville, FL 32611-0690, USA
e-mail: brath@ufl.edu

C. Kole (ed.), *Genomic Designing of Climate-Smart Vegetable Crops*,
https://doi.org/10.1007/978-3-319-97415-6_5

Peppers belong to the genus *Capsicum* in the Solanaceae family and are native to tropical and temperate Americas. Members of the *Capsicum* genus have unique botanical traits compared to their sister genus *Lycianthes* by having longitudinal slits in the anthers, nectary in the flower, base chromosome numbers as $x = 12$ or 13, and pungent alkaloids in the fruit (Carrizo Garcia et al. 2016). A molecular phylogenetic study revealed 11 clades in this genus, namely, Andean, Annuum, Atlantic Forest, Baccatum, Bolivian, Caatinga, Flexuosum, Longidentatum, Purple Corolla, Pubescens, and Tovarii (Carrizo Garcia et al. 2016). Among them, the fruit of 5 species of *Capsicum* belonging to Annuum, Baccatum, and Pubescens clades make up the economically important vegetables and spices of commerce. Key morphological traits to distinguish the different cultivated species are listed in Table 5.1. Most plant breeding efforts have been focused on *C. annuum* and *C. frutescens*.

Multiple studies on domestication of *Capsicum* species revealed the locations of highest genetic diversity. The probable places of origin of these crops are Ecuador, Peru, Bolivia, and Mexico (Pickersgill 1997; Kraft et al. 2013; Silvar and Garcia-Gonzalez 2016). The Andean and the Atlantic forest clades of *Capsicum* have multiple wild species distributed in different habitats and climatic zones, and they represent a gene pool of potential utility for improving domesticated Capsicums (Jarret et al. 2019).

Multiple varietal types of peppers are known in commercial trade (Govindarajan and Salzer 1985, Crosby 2007; Table 5.2). Among them, the bell peppers make up the major tropical vegetable worldwide. The first description of bell pepper cultivation is in 1774 (Boswell 1937). Pepper types that are pungent, referred in this article as "specialty peppers" or "chili" are popular for specific uses as condiments, and spices for flavoring culinary dishes. Table 5.3 lists some of the popular types of specialty peppers. Many of these are landraces, often grown in specific regions of the world and are used for specific culinary purposes.

5.2 Prioritizing Climate-Smart Traits

The Intergovernmental Panel on Climate Change's (IPCC) Fifth Assessment Report (AR5) has outlined unequivocal evidence for global warming of the climate system and increasing trend of anthropogenic CO_2 emissions (IPCC 2014). It is expected that extreme weather events such as high temperature stress, freezing, drought, and flooding will negatively affect future vegetable production systems. These events will also affect the pests, diseases, and beneficial organisms in the agroecosystems. Hence, specific efforts are needed to (a) breed crop varieties adapted to the changing climate, (b) use diverse genetic resources of the crop to guard against failures and (c) employ breeding methods and cultivation practices that together maximize sustainability and productivity. The objective of this review is to examine the potential for breeding Capsicum varieties improved for fruit yield, quality, and tolerance to abiotic and biotic stress, so that sustainable production of vegetables will become possible even under changing climatic conditions. This article aims to illustrate specific points on

Table 5.1 Clade, chromosome number, genome size, place of domestication, and key traits for the five species of Capsicum that are used for food (NA = Not available)

Species	Clade	Chromosome number (2n)	Genome size (Gb)	Place of domestication	Key traits
C. annuum	Annuum	24	3.5	Mexico, Bolivia	White or dingy white petals, blue to purple anthers, solitary flowers at node, smooth yellow seeds
C. chinense	Annuum	24	3	Peru	White to waxy yellowish petals, blue to purple anthers, 3–5 pedicles per node, erect, seed margins wrinkled, yellow seed, distinct constriction at the base of the calyx
C. frutescens	Annuum	24	NA	Ecuador	White to waxy yellowish petals, blue to purple anthers, two pedicles per node, erect, smooth yellow seed
C. baccatum	Baccatum	24	3.2	Bolivia, Argentina, and Brazil	White petals with yellow or tan markings at base, yellow anthers, pedicels one or rarely two per node, calyx serrated, yellow smooth seed, giant cells in the mesocarp
C. pubescens Ruiz et Pav.	*Pubescens*	24	NA	Bolivia and Columbia	Violet corolla, white at base, blackish-brown wrinkled seeds, pubescent stem and leaves, low temperature tolerance

Table 5.2 Horticultural varietal groups of peppers, their key traits, and common varieties in that type

Horticultural varietal group	Key fruit traits	Common variety/type name
Bell group	Fruit blocky, large, 3–4 lobed, dark green turning red when ripe, generally sweet, or mildly pungent	Sweet bell
Tomato group	Fruit tomato-like, flattened, and four-lobed	European Sweet Peppers
Pimiento group	Fruit short, conical, thick red pericarp	Pimiento
Cayenne group	Fruit slim, pointed, slightly curved	Cayenne, Anaheim, New Mexico
Tabasco group	Fruit slim, tapered, very pungent	Tabasco
Cherry group	Fruit globose, three-lobed, upright fruit sweet to pungent	Red Cherry
Celestial group	Fruit cone-shaped, multiple colors upright	Ornamental

this topic citing select and recent publications instead of a comprehensive historical review of the literature.

5.2.1 Fruit Yield

Many modern cultivars of green bell peppers have been optimized for high yields when grown under field conditions with drip irrigation and plastic mulch. In the United States, pepper for the fresh vegetable market is harvested manually. Under ideal field conditions, marketable yields range about 30–40 t ha^{-1} (Locascio and Stall 1994). In more recent field trials that tested several improved varieties, more average yields have been realized, but a greater degree of varietal differences and seasonal variations for fruit yield have been observed (Sezen et al. 2006; SWREC 2019).

Aspects related to fruit yield of peppers have been studied under controlled environment growth chambers, greenhouse hydroponics, and field conditions. Some of these studies led to models to predict the fruit yield especially under greenhouse production (Lin and Hill 2008; Lin and Dietmar 2009). Yield reductions due to deficit irrigation, high temperature stress (Pagamas and Nawata 2008) and soil salinity have been documented. Certain studies suggested that fluctuations in fruit yield can be reduced by inducing parthenocarpy via auxin application (Heuvelink and Korner 2001). The role of gibberellin in preventing flower and fruit abscission in pepper is well known (Tiwari et al. 2012) and it was suggested that application of growth

Table 5.3 Types of specialty peppers and their characteristics. This table is not exhaustive as there are numerous more specialty types in use

Specialty type	Remarks
Anaheim	A mild variety of the cultivar "New Mexico No. 9". Pungency: 500–2500 Scoville units
Bird's Eye	Very pungent fruit. Sometimes referred to as "Piri piri". Pungency: 50,000–100,000 Scoville units. Used in sauces and in Asian recipes of soups, salads, and stir-fries
Cayenne	Moderately pungent pepper, pungency: 30,000–50,000 Scoville units. Used dried
Friggitello	Also known as Greek golden pepperoncini, slightly pungent peppers, used fresh or pickled
Guajillo	Dried form of the mirasol chili, a landrace variety of *C. annuum*. Used in Mexican cuisine such as for salsa for tamales, sauces, and spice rubs
Habanero	*C. chinense*. Fruit blocky, very pungent like Scotch Bonnet. Fruity flavor
Hatch	Chile grown in the Hatch Valley, New Mexico, USA; green and red chilies are used for their unique flavors. Used for New Mexican chiles rellenos. Pungency: 300–70,000 Scoville units
Jalapeno	Hot pepper used green or red in Mexico and Southwestern U.S., used for salsa, pickles, stuffed peppers, jelly and for flavoring as smoked peppers. Ripe jalapeno that has been dried is referred as chipotle. Pungency: 800−3500 Scoville units
Malagueta	*C. frutescense* pepper from Brazil used for sauces. Pungency 60,000–100,000 Scoville units
Paprika	Medium length pepper for producing chile powder. Hungarian types used for mechanized production
Poblano	A mild dark green pepper from Puebla, Mexico. Also known as Ancho when dried. Poblano is used for stuffed fresh and roasted and in chiles rellenos poblanos
Pueblo	Chiles cultivated by the Puebloan peoples of New Mexico
Rio Grande	Hot peppers grown in the Rio Grande region, New Mexico
Rocoto	*C. pubescens* native to Peru. In Bolivia, they are known as "locoto". Used for stuffed peppers and in rocoto rellenos
Scotch Bonnet	Native to the Caribbean islands and Central America. Pungency: 80,000–400,000. To add flavor to Caribbean and West African cuisine
Serrano	A chile pepper from the mountainous regions of Mexico used in Salsa. Pungency: 10,000–23,000 Scoville units
Shishito	Fruit sweet with occasional pungency. This variety originates from Japan. Wrinkled fruit is thin walled, harvested green and used skewered and broiled or pan-fried in oil
Tabasco	A pepper from Tabasco state, Mexico. Pungency 30,000–50,000 Scoville units. Used for Tabasco sauce and peppered vinegar
Yellow Wax	Light yellow, waxy when immature. Fruit sweet to high pungency. Used for pickles

regulators may be a way to improve the yield and quality of peppers (Belakbir et al. 1998; Maboko et al. 2015). Genetic studies for selectable traits altering plant growth regulators are needed to incorporate them into efforts to breed for increased yield.

A multilocation trial of ten lines of peppers in five countries evaluated yield components and found high influence of environment on yield. Genotype was the greatest contributor to variability in yield components and the authors suggested four accessions for improving yield stability in breeding programs (Barchenger et al. 2018). Others have tested potential correlations between multiple fruit and plant traits to fruit yield. Ramalho do Rego et al. (2011) showed that in *C. baccatum* landraces fruit width, fruit weight, and fruit dry matter were correlated to yield. Singh et al. (2009) analyzed yield-related traits in thirty genotypes of chile peppers and found high heritability and genetic advance for fresh and dry fruit yield per plant, fruit weight, fruit diameter, and the contents of oleoresin and capsaicin.

Certain genetic studies focused on identifying the chromosomal regions that were responsible for genetic differences in fruit yield among accessions. Linkage maps are powerful tools for discovering loci controlling complex traits via identification of quantitative trait loci (QTLs). Information obtained from biparental mapping populations could be useful to explain candidate genes underlying natural genetic variation for the trait of interest. Barchi et al. (2009) analyzed a population of 297 recombinant inbred lines (RILs) derived from a large-fruited "Yolo Wonder" and the small-fruited chile "Criollo de Morelos 334". Several highly significant QTLs for fruit traits related to yield were recognized including for flowering earliness, fruit weight, fruit length, and fruit diameter (Barchi et al. 2009). Lu et al. (2012) built a linkage map using 458 molecular markers including single nucleotide polymorphism (SNP) markers, agronomic, and morphological markers to characterize 23 QTLs for 11 traits. Out of these, the most significant QTL was on linkage group 5 that explained >90% of phenotypic variance for resistance to the pathogen *Phytophthora capsici*.

5.2.2 Hybrid Production

Many studies have shown hybrid vigor for both fruit yield and quality traits (Geleta et al. 2004; Bhutia et al. 2015) and determined combining ability for yield and fruit quality using diallel analysis (Nascimento et al. 2014). In sweet bell peppers, F_1 hybrids had significantly lower "days to 50% flowering" and "days to first picking" and significantly greater plant height, harvest duration, fruit yield per plant, fruits per plant, marketable fruit per plant, fruit length, and pericarp thickness compared to the parents (Sood and Kumar 2010). For producing F_1 hybrid varieties efficiently, researchers studied both genic male sterility (GMS) and cytoplasmic genic male sterility (CGMS) systems. Recessive single-gene mutations including *ms-1* and *ms-2* leading to male sterility have been identified (Shifriss 1997, for a review). Seed companies use the genic mechanism *msms* on a large scale to produce hybrid sweet bell pepper. In the genic male sterile system, the male sterile line (*msms*) is maintained by pollinating with *Msms* (male fertile) line. Fifty percent of the F_1 will be male fertile

and the other 50% male sterile and the seeds are collected from male sterile (*msms*) plants only, after identification. In the hybrid seed production block, male fertile plants in the female row are removed and the seed harvested from male-sterile plants are hybrid seeds (Dhall and Cheema 2010).

In CGMS, a male sterile line carrying a maternally inherited cytoplasmic male sterility (CMS) is used as a seed parent line. There will be no need to prevent self-pollination via emasculation. A line carrying a restorer-of-fertility (*Rf*) nuclear gene is used as the pollen parent (Dhall and Cheema 2010). The resulting male fertile F_1 plant has male fertility due to the action of *Rf*. Since the first report of CMS in an Indian accession (USDA PI 164835) (Peterson 1958), multiple sources of male-sterile germplasm and fertility restorers have been identified. Asian Vegetable Research and Development Center (AVRDC), Taiwan has released two CGMS lines of chile CCA-4759 and CCA-4757 that are widely used for hybrid seed production and for deriving temperature-stable CMS sources of male sterility via breeding (Meena et al. 2018).

Progress has been made in understanding the nature of both male sterility and restorer genes. Kim et al. (2007) reported that a novel open reading frame (ORF) termed orf456 was responsible for male sterility by expressing it in transgenic Arabidopsis plants. A study on the transcriptomes and proteomes of genic male sterility showed that 52 genes and their protein products were differentially expressed between male fertile and male-sterile plants (Cheng et al. 2019). Another study that examined the mitochondrial genome sequences identified 35 ORFs as potential candidate genes for male sterility (Wang et al. 2019). In a study by Sun et al. (2016) a morphological marker and two sequence-characterized amplified region (SCAR) markers, were shown to identify lines expressing cytoplasmic male sterility. Fine mapping of restorer-of-fertility in pepper identified a candidate gene encoding a pentatricopeptide repeat (PPR)-containing protein (Jo et al. 2016). Molecular markers to identify fertility restorer genes have been reported (Kim et al. 2006; Lee et al. 2008) and the applicability of these markers in certain breeding populations is very good.

5.2.3 *Fruit Quality Traits*

The standards for fruit quality traits differ for different commodity types of peppers. Sweet bell peppers are graded according to multiple fruit characteristics. United States Department of Agriculture grading standard's highest grade of "fancy" is defined as mature green color, similar varietal characteristics to others in the box (uniformity), firm (not shriveled, soft, or pliable), well-shaped, at least 3 in. in diameter and 3.5 in. long, and if not green, fruit showing color specified on container. Also, this grade of peppers should be free of sunscald, freezing injury, and decay. U.S. No. 1 and U.S. No. 2 are the next lower categories of grades and such grades are defined for chile peppers also (USDA 2019). Minimal standards are set for scars, sunburn, bacterial spot, hail, and other injury in each of these categories.

In the Solanaceae, some species produce capsules (e.g., tobacco and petunia) and others produce fleshy berries (e.g., tomato and pepper). The fruits of fleshy berries are characterized by an abundant amount of collenchyma, an increased number of cell layers, and a parenchymatous endocarp often expanding into the locules (Pabon-Mora and Litt 2011). Because pepper pericarp makes up most of the edible portion of the fruit, it is essential to focus on the traits related to the development and quality of this tissue. The pericarp tissue's physical properties, water content and metabolite concentration, and composition all define the qualities that together determine the end uses and value of the commodity. For this reason, many studies have explored the nature of fruit-related traits, their inheritance, and biochemistry.

While many of the fruit quality traits are largely controlled by multiple genes, environmental factors significantly influence specific aspects of fruit quality. A study on the characterization of postharvest water loss in ripe fruit during storage found that total cuticle wax amount, lipoxygenase activity, and cell membrane ion leakage were directly related to fruit postharvest water loss rate during storage (Kissinger et al. 2005). One could expect reductions in pre-harvest fruit quality due to global climate change as increased problems of sunburn, bacterial spot, and injury could occur under less than ideal environmental conditions.

5.2.3.1 Pericarp Thickness and Texture

In sweet bell peppers, fleshy pericarp (3 mm to 6 mm thickness) is preferred but in certain cayenne peppers, thin pericarp (0.5–2 mm) capable of rapid drying may be critical for processing the pepper into paprika powders. Pericarp thickness also affects cuticular cracking, a defect influenced by limitation of night transpiration by high humidity or low temperature that increases the turgor potential of the pericarp cells (Aloni et al. 1998). In the Solanaceae, young fruit contain cells that are predominantly 2C and 4C but during fruit development endoreduplication (i.e., DNA replication without nuclear and cell division) increase the DNA content of nuclei to several 100s of C, a phenomenon referred to as polysomaty. A flow-cytometric study of 12 accessions revealed that there was a positive correlation ($r = 0.88$) between pericarp thickness and polysomaty in ripening fruit (Ogawa et al. 2010). Since pericarp thickness is often correlated to fruit fresh weight and diameter (Ogawa et al. 2010; Oliveira Vilarinho et al. 2015), during domestication, selection for larger sized fruit could have resulted in fruit with thicker pericarp.

Ben-Chaim et al. (2001) reported a study where a linkage map using morphological and restriction fragment length polymorphism (RFLP), and random amplified polymorphic DNA (RAPD) markers, was developed. This study identified multiple quantitative trait loci for fruit-related traits including pericarp thickness. Our study on fruit of an inbred with thin pericarp and an inbred with thick pericarp suggested that thick pericarp trait is due to both increased number of cell layers in the pericarp and larger cells (Oliveira Vilarinho et al. 2015).

Few studies examined texture related traits in pepper fruit. Sensory analyses on fresh sweet peppers scored for traits such as stickiness, toughness, crunchiness,

and juiciness of the fruit (Eggink et al. 2012) are available, but quantitative measures related to texture are limited (Cheng et al. 2008). Mutations in tomato such as *ripening-inhibitor* (*rin*), *non-ripening* (*nor*), *never-ripe* (*nr*), and *colorless non-ripening* (*cnr*) have pleiotropic effects on fruit texture (Seymour et al. 2002), and hence, their homologs in *Capsicum* could be tested for their potential role in fruit texture in pepper.

5.2.3.2 Fruit Shape and Size

Capsicum germplasm contains a wide range of fruit shape and size variations (Wang and Bosland 2006). During the first stage of anthesis and fruit set, old fruit have an inhibitory effect on the growth of younger fruit (Ali and Kelly 1992). Hence, it was suggested that maintenance of vigorous growth from flower bud formation to flower development is critical to reduce variations in fruit size on the upper nodes of the plant (Ali and Kelly 1992).

The earliest studies on the inheritance of fruit shape in peppers recognized the involvement of multiple heritable factors in controlling this complex trait (Kaiser 1935; Ben-Chaim et al. 2013). It was hypothesized that non-deciduous fruit that remained on the plant until harvest and the change in position from erect to pendant fruit were both selected during domestication (Paran and Van der Knaap 2007). These authors reasoned that these changes likely led to an increase in fruit size and provided better protection from sun exposure, and predation by birds. In support of their idea, wild peppers have fruits that were oval, spherical, or elongated, but greater diversity of shapes exist in selected varieties.

Among the many quantitative trait loci known for fruit size and shape, some of them—eight QTLs for fruit weight, five QTLs for pericarp thickness, and one for fruit shape—were found in common genomic regions between tomato and pepper (Ben-Chaim et al. 2006). The *ovate* locus in tomato was shown to be responsible for the pear-shaped elongated fruit shape in tomato in multiple genetic studies. The genetic analysis of elongated fruit shape in pepper led to the identification of two major QTLs *fs3.1* and *fs10.1* (Ben-Chaim et al. 2001, 2003; Rao et al. 2003). But most QTLs for pepper fruit shape were not common to QTLs identified for fruit shape in tomato, likely due to structural and developmental differences between tomato and pepper (Paran and Van der Knaap 2007). In contrast to these, Tsaballa et al. (2011) cloned ovate-like genes from pepper varieties that had round or long fruit shape. No significant structural differences were detected for the genes from the round and long fruited types, but the expression of *CaOvate* was significantly different between them. Downregulation of this gene via virus-induced gene silencing altered "round" fruit type into "elongated" fruit type (Tsaballa et al. 2011) suggesting that *CaOvate* is a candidate gene for fruit shape in pepper.

Natural fruit shape variations in *Capsicum* can be used to develop varieties with novel shapes to increase consumer interest. Certain fruit shapes may not be good for large-scale processing. For example, corrugated fruit shape (Oliveira Vilarinho et al. 2015) likely controlled by two recessive genes, is attractive but could trap debris

from the field during harvest. Fruit shape could also have an influence on postharvest quality. A study on 24 accessions of *C. chinense* found that fruit volume and width were positively correlated to improved shelf life (Elibox et al. 2017).

5.2.3.3 Total Soluble Solids, Sugars, and Organic Acids

Total soluble solids can be measured in fruit extracts using refractometry and the value, measured in Brix, is a proxy for the concentration of sugars, organic acids, and other soluble substances in the fruit. Like in tomato, in peppers this trait has a low narrow-sense heritability (Ben-Chaim and Paran 2000) suggesting that selections for this trait will be a challenging task for plant breeders. In a study on fruit quality, Eggink et al. (2012) analyzed the sugars and acids in fresh peppers harvested from 24 varieties. While sucrose concentrations were below the detection limit (0.003 g/g fwt), glucose and fructose were in approximately equal amounts (0.03 g/g fwt). Among organic acids, citric acid was the most abundant (1.9–6.1 mg/g fwt), followed by ascorbic acid (1.4–2.5 mg/g fwt) and malic acid (0.1–1.6 mg/g fwt) (Eggink et al. 2012). Metabolomic studies in developing fruit showed that citrate and dehydroascorbate levels dramatically increase during early developmental stages followed by small reductions during ripening stages (Osorio et al. 2012). This study found malate levels decreased during later developmental stages but increased during ripening when the genes related to sucrose degradation were upregulated while starch synthesis genes were down-regulated (Osorio et al. 2012). It has been suggested that organic acids in fruits especially citrate and malate, may supply substrates for respiratory processes of the fruit (Batista-Silva et al. 2018).

Studies have documented a great deal of genetic variation for the concentrations of sugars and organic acids in Capsicum (Rosado-Souza et al. 2015) and hence selection for specific metabolites is a good breeding strategy to influence fruit quality. Ascorbic acid can be synthesized by multiple routes—from D-glucose-6-phosphate of glycolysis, from oxidation of myo-inositol and from breakdown of pectin. Studies in tomato reveal that it may be possible to increase the levels of ascorbic acid and sugars via modifying specific steps related to their biosynthetic processes (Batista-Silva et al. 2018; Rigano et al. 2018). These strategies are likely applicable in pepper also, although pepper as a non-climacteric fruit differs from tomato in ethylene-regulated processes (Batista-Silva et al. 2018).

The sensation of sweet taste (described in taste panels as fruity/apple taste) is also influenced by volatile compounds. In peppers, volatiles p-menth-1-en-9-al, (E)-β-ocimene, (Z)-2-pentene-1-ol, and (E)-geranylacetone were reported to be positively correlated to sweet taste (Eggink et al. 2012). The concentrations and composition of sugars and organic acids and volatiles that influence their perception are important determinants for fruit taste. Hence, their inheritance and candidate genes controlling their concentrations need to be identified so that informed decisions could be made during crop improvement.

5.2.3.4 Capsaicin Content

Capsaicinoids are the alkaloids responsible for the pungency of chile peppers. Pungency has likely evolved as a deterrent for seed predators, pests, or pathogens. Studies feeding capsaicin to larvae of Noctuidae support the notion that certain insects were deterred while other species have adapted to its host (Ahn et al. 2011). Exogenous capsaicinoids (capsaicin and *N*-vanillylnonanamide) have been shown to be effective in inducing host defense against fungal pathogens such as *Verticillium dahlia* and *Botrytis cinerea* (Velosco et al. 2014).

Two major pungent compounds accumulated in chiles are capsaicin and dihydrocapsaicin. The levels of capsaicinoids are controlled by genes, but are influenced by several environmental factors, which have been known from numerous studies. The reported values for capsaicin concentration are also affected by the nature of the sampling and extraction methods and analytical methods for quantification. Canto-Flick et al. (2008) examined the levels of capsaicinoids in 18 *C. chinense* Habanero accessions and the levels in different varieties ranged from 10 mg/g to 60 mg/g fwt.

Since the first study of inheritance of pungency trait (Deshpande 1935), several genetic investigations identified additional loci. Blum et al. (2003) used mapping methods to identify a QTL for capsaicin content on chromosome 7. A major QTL termed *cap* explained 34–38% of phenotypic variation. Ben-Chaim et al. (2006) identified six QTLs controlling capsaicinoid levels including confirmation of the major QTL identified in Blum et al.'s (2003) study. Capsaicin synthetic pathway is shown in Fig. 5.1 and it is derived from two amino acid precursors, phenylalanine and valine. Baas-Espinola et al. (2016) tested whether these two amino acids synthesized in the fruit in situ act as precursors for capsaicin. Their tests showed that when either amino acid synthesis was blocked via supplied inhibitors, capsaicin levels decreased (Baas-Espinola et al. 2016). The final step in capsaicin synthesis is the enzymatic

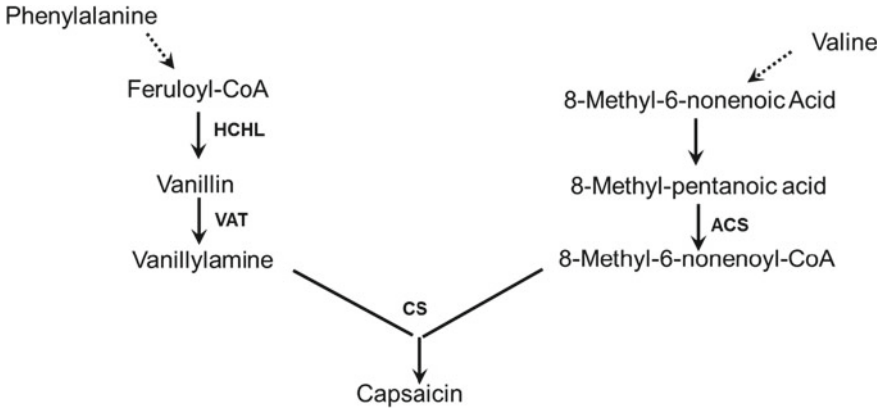

Fig. 5.1 Capsaicin biosynthesis in peppers. HCHL—hydroxycinnamoyl-CoA hydratase/lyase; VAT—vanillin aminotransferase; CS—capsaicinoid synthase (acyltransferase). Dotted lines represent multiple steps not shown here

condensation of vanillylamine and medium-chain length fatty acids. The condensing enzyme capsaicinoid synthase acts on the medium-chain length fatty acyl CoA and requires Mg^{2+} and ATP (Thiele et al. 2008).

Mazourek et al. (2009) accomplished cloning of targeted transcripts potentially involved in capsaicinoid biosynthesis. The proteins coded by these transcripts were tested for their subcellular localization and the genes were placed in a linkage map (Mazourek et al. 2009). Han et al. (2018) have used a combination of QTL mapping and genome-wide association studies (GWAS) to identify QTLs for capsaicin content. Out of sixty-nine QTLs for capsaicinoids identified in GWAS ten were also identified in the study using biparental mapping population. This study identified five candidate genes for capsaicinoid content: pAMT, C4H, CSE, 4CL from phenylpropanoid pathway, and FatA (Han et al. 2018). Among the four gene products identified in the phenylpropanoid pathway, the function of CSE is unclear, while the functions and subcellular locations for others are known. The FatA regulates the chain length of the fatty acids (Han et al. 2018). There are several lines of evidence to support the idea that capsaicinoid biosynthesis is regulated by R2R3-MYB transcription factor CaMYB31 (Arce-Rodriguez and Ochoa-Alejoa 2017). Two other ERF family transcription factors have been reported to influence the accumulation of capsaicinoids in the placenta of peppers based on their correlated expression in the fruit accumulating capsaicin (Keyhaninejad et al. 2014).

Metabolomic studies on developing fruit show that in the early stages of fruit development glycosides of luteolin, apigenin, and quercetin, shikimic acid, gamma-aminobutyric acid, and putrescine were abundant. These compounds gradually decreased at later stages when there was a significant increase of several amino acids, capsaicin, dihydrocapsaicin, and kaempferol glycosides (Jang et al. 2015). Virus-induced gene silencing has been used to test the functions of three genes potentially involved in capsaicinoid accumulation in pepper fruits (Abraham-Juarez et al. 2008).

5.2.3.5 Fruit Coloration and Carotenoids

Pepper fruit coloration at maturity is determined by the accumulation of carotenoids during fruit ripening. In varieties that have the ability to accumulate anthocyanins, immature, or mature fruit color could develop violet or black or purple as anthocyanin pigments accumulate often at the same time when changes in carotenoid composition happen. Immature green pepper turns color during ripening and the fully ripened fruit is red in most varieties. In some of the varieties, mature fruit colors are yellow or orange and rarely white.

Pepper carotenoids are nutritionally valuable especially beta-carotene which is a precursor for retinol (vitamin A) and lutein and zeaxanthin which are important antioxidants for eye health (Abdel-Aal et al. 2013). Because of the nutritional significance of carotenoids, multiple studies have measured total carotenoid levels and composition in different varieties of peppers.

The biosynthetic pathway to carotenoids and xanthophylls in pepper fruit is shown in Fig. 5.2. In red-fruited varieties, ripe fruit contains about 9–54% of the total carotenoids as the red carotenoid capsanthin (Antonio et al. 2018). β-Carotene levels ranged from 0.9 to 21%, lutein 0–13.4% and zeaxanthin 0.5–25.54% of total carotenoids, respectively (Antonio et al. 2018; Wall et al. 2001). Wahyuni et al. (2011) analyzed ripe fruit from 32 accessions of *Capsicum* for carotenoids, flavonoids, ascorbic acid, vitamin E, and capsaicinoids. The authors recognized the presence of carotenoid fatty acyl esters but did not quantify the esterified forms of carotenoids (Wahyuni et al. 2011). Brown-fruited types contained chlorophyll b and lutein along with carotenoids. Some of the yellow-fruited accessions had little capsanthin, but contained higher concentrations of lutein (0.08 mg/g) than the red accessions (Wahyuni et al. 2011).

In the 80s, geneticists have used the mature fruit color as a qualitative trait to study inheritance of the character by crossing yellow or orange-fruited line with red-fruited line (Hurtado-Hernandez and Smith 1985). By comparing genetic linkage groups of pepper with that of tomato, Thorup et al. (2000) identified ten structural genes from the Capsicum carotenoid biosynthetic pathway. This study, a nice example for a comparative structural genomic approach for candidate gene discovery, identified loci corresponding to capsanthin–capsorubin synthase (Ccs), the B locus corresponding

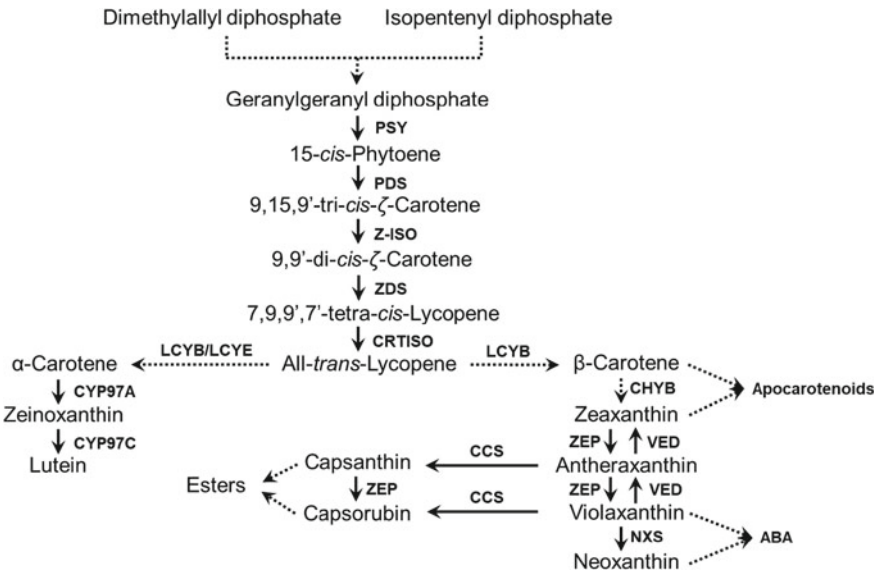

Fig. 5.2 Carotenoid biosynthesis in peppers. PSY, phytoene synthase; PDS, phytoene desaturase; Z-ISO, ζ-carotene isomerase; ZDE, ζ-carotene desaturase; CRTISO, carotenoid isomerase; LCYE, lycopene ε-cyclase; LCYB, lycopene β-cyclase; CYP97A, CYP97A-type β-ring hydroxylase; CYP97C, CYP97C-type ε-ring hydroxylase; CHYB, β-carotene 3-hydroxylase; ZEP, zeaxanthin epoxidase; VED, violaxanthin de-epoxidase; NXS, neoxanthin synthase; CCS: capsanthin–capsorubin synthase. Dotted lines represent multiple steps not shown here

to β-carotene accumulation in tomato, and lycopene ε-cyclase, corresponding to *lutescent-2* mutation in tomato (Thorup et al. 2000).

There are multiple steps in the carotenoid synthetic pathway in which mutations can lead to orange or yellow fruit color. Provitamin A carotenoids are yellow-colored while capsanthin and capsorubin are red. It appears that the synthesis of the red pigment drive a high flux through this pathway during ripening. So, in all red-fruited accessions, an inverse relationship was found between total carotenoid content and the red to yellow isochromic pigment fraction ratio (R/Y) and the capsanthin-to-zeaxanthin ratio (Hornero-Mendez et al. 2000). Ha et al. (2007) showed that structural mutations in capsanthin–capsorubin synthase gene can lead to yellow ripe fruit color. They also showed that the red fruit accumulated more total carotenoids than yellow accessions, but certain yellow-fruited accessions had exceptionally high lutein levels. Guzman et al. (2010) showed that certain orange-fruited accessions are more suitable as sources of gene to achieve high provitamin A levels in the ripe fruit.

Fibrillin, a chromoplast-specific protein, suggested to have a role in carotenoid storage, was shown to coexpress with carotenoid biosynthetic enzymes during fruit ripening (Chen et al. 1998; Kilcrease et al. 2015). The gene encoding this protein was induced in the leaves of wounded or drought-stressed plants (Chen et al. 1998) indicating additional roles for this protein in stress protection. Vidi et al. (2006) reported that plastoglobule, the lipoprotein particle in plastids is the site of tocopherol cyclase and tocopherol (vitamin E) accumulation.

Violet or black pigmentation on leaves, flowers, and fruits found in certain pepper accessions are due to the accumulation of anthocyanins especially delphinidin. This trait is affected by numerous environmental factors such as light stress and temperature (Lightbourn et al. 2007) and there is special interest in breeding vegetables with high levels of anthocyanins due to their potential benefits as health-promoting phytochemicals. There is increasing evidence for the control of the synthetic pathway via Myb transcription factors (Stommel et al. 2009).

Wahyuni et al. (2014) identified quantitative trait loci for the accumulation of flavonoid metabolites. Together with efforts to integrate metabolomics and molecular markers (Wahyuni et al. (2013), marker-assisted selections for high flavonoid levels fruit will be very useful to breed peppers with high nutritional quality. Liu et al. (2018) has presented an overview of the biosynthesis and degradation mechanisms for anthocyanins in the fruit of Solanaceous vegetables tomato, pepper, and eggplant. The wealth of knowledge available on the biochemistry and genetics of fruit colors indicates that there are excellent opportunities to breed for highly nutritious pepper varieties with novel and consumer-preferred colors.

5.2.4 Heat Stress Tolerance

Although peppers are adapted to tropical and subtropical climates, the crop is negatively affected by the high radiation and higher than optimal temperatures during the spring–summer harvesting periods. Like in many crops, certain stages of the

reproductive phase are more sensitive to high temperature stress than the vegetative phase of development (Pagamas and Nawata 2008). Under high temperature stress, there was an increase in abscission of flowers and fruit and it coincided with an increase in ethylene (Huberman et al. 1997). A study focused on testing the effect of high temperature stress (33 °C for 120 h) on flowering bell pepper plants found that flower buds at <2.5 mm in length and flowers that reached anthesis during the high temperature exposure had reduced fruit set (Erickson and Markhart 2002). It was suggested that under high temperature stress the pollen's acid invertase activity is reduced and the levels of sucrose and starch were higher than pollen from control plants (Aloni et al. 2001). Interestingly increased CO_2 counteracted the negative influence of high temperature stress (Aloni et al. 2001). The negative effects of high temperature stress are likely due to direct effects of the high temperature than temperature stress-induced water stress (Erickson and Markhart 2001). Other than the negative effects on fruit set, high temperature stress can also reduce the stability of disease resistance genes and the activity of pollinators. Tomato spotted wilt virus resistance in peppers conferred by *Tsw* gene is less stable at 32 °C than at 22 °C (Moury et al. 1998). Under high soil temperatures, the southern root-knot nematode resistance conferred by the *N* gene was not sufficient to protect the plants from nematode damage (Thies and Fery 1998).

Cultural practices and choice of the planting date can help reduce the problems of high temperature stress during flowering and fruit set. Shading of the crop has been found to be good for reducing sun-scalded fruit in greenhouses. Effects comparable to shading were achieved using grafted plants with specific rootstocks (Lopez-Marin et al. 2013; Ropokis et al. 2019), suggesting the viability of breeding specific rootstocks to improve stress tolerance.

Several studies have focused on identifying genes for heat stress tolerance in pepper and tested them in transgenic model systems. For example, Guan et al. (2018) characterized CaHSL1 a protein kinase involved in protecting plants from high temperature stress under high humidity. Isbat et al. (2009) identified a *BAX inhibitor-1*, a gene associated with regulation of programmed cell death endowed transgenic plants overexpressing it tolerance to multiple stress factors. Despite the recognition of genetic variability for heat stress tolerance (Reddy and Kakani 2007; Guo et al. 2015) and specific molecular studies on the function of heat shock proteins (Guo et al. 2016; Sun et al. 2019), studies probing the inheritance of heat tolerance as a quantitative trait are not available in pepper. Future plant breeding efforts are needed to develop cultivars improved for high temperature stress tolerance.

5.2.5 Cold Stress Tolerance

As a tropical crop, peppers are frost-sensitive. Multiple studies focused on documenting the negative effects of chilling stress on various metabolic processes in peppers (Tijskens et al. 1994). For example, Mercado et al. (1997) compared a temperature regime of 29 °C day/20 °C night to a regime of 25 °C day/14 °C night. The plants

grown at the lower night temperature showed an improved chilling resistance when exposed for 4 nights at 6 °C (Mercado et al. 1997). Low night temperatures (14 °C or lower) have negative influences on pollen viability, number of pollen, and functioning of female organs of the flower (Pressman et al. 1998; Cruz-Huerta et al. 2011). Airaki et al. (2012)'s study showed the role of reactive oxygen species in cold stress-induced damage. Genotypic effect on low temperature tolerance is known (Pressman et al. 1998), but selections for this trait have not been reported.

Peppers are susceptible to cold injury when the harvested fruit are stored in the cold (7 °C) for extended periods of time. Chilling injury manifests as spots of surface pitting. It is possible to improve cold storability using low temperature conditioning combined with the application of methyl jasmonate (Wang et al. 2019) and UV-C treatments (Vicente et al. 2005). A proteomic study found that chilling-stressed bell peppers had higher ethylene production, changes in sugar and organic acids, and significant alternations in proteins involved in redox homeostasis and carbohydrate metabolism (Sanchez-Bel et al. 2012).

5.2.6 Salinity Tolerance

Substantial genotypic variation for salinity tolerance is known in Capsicum (Chartzoulakis and Klapaki 2000; Aktas et al. 2006; Niu et al. 2010; Bojorquez-Quintal et al. 2016). In the study by Aktas et al. (2006), the sensitive varieties accumulated significantly greater sodium ions in their shoot than the resistant varieties. The salt-tolerant varieties exhibited lower declines in relative water content and increased levels of enzymatic antioxidants (Aktas et al. 2012). Fruits were more sensitive to salinity than leaves and stems (Azuma et al. 2010) and the negative effects of salinity on ascorbic acid synthesis were suggested to be an important factor. Abscission of leaves in response to salinity stress is likely in response to increased ethylene, which in turn leads to hydrogen peroxide production in the abscission zone (Sakamoto et al. 2008). Under salinity stress, increased reactive oxygen species induce blossom-end rot disorder that likely aggravates calcium deficiency (Rubio et al. 2009; Saure 2014).

Several mitigation methods for dealing with high salinity has been tested and found to be somewhat useful in pepper production. These cultural methods involve better nutrient management such as improving calcium nutrition to reduce damage by sodium or chloride, use of protectants such as glycine betaine or catechin, and better irrigation methods. Rootstocks have been identified to generate saline tolerant grafted plants (Guiffrida et al. 2013; Penella et al. 2016, 2017). Molecular biology research has led to the identification of several genes that have multiple roles in salinity (and other abiotic stress tolerance) and pathogen defense in peppers (Do et al. 2004; Kim and Hwang 2014, for examples).

5.2.7 Resistance to Diseases and Pests

Pepper crop is affected by numerous diseases. Hence, prudent use of fungicides, miticides, and insecticides is important to effectively control the pests and pathogens and obtain optimal yield and fruit quality. However, integrating the use of resistant varieties is an environmentally sound approach for pest management. Identification and use of major genes for disease resistance in the crop has become an important activity in every crop breeding program. Races of the pathogen continuously evolve at different rates to break the host resistance—a major problem for breeders aiming to develop resistant varieties. One approach to this problem is to pyramid different resistance genes with differing modes of action together in one line. Palloix et al. (2009) demonstrated that polygenic resistance to *Potato virus Y* in pepper was superior to monogenic resistance. Information regarding high or moderate resistance or susceptibility of pepper germplasm to some of the major diseases and the nature of inheritance of the resistance trait are available (for examples, Lee and Kim 2012; Naegele et al. 2017).

Bacterial leaf spot caused by *Xanthomonas* spp. is a major problem in pepper and tomato production worldwide. *Xanthomonas campestris* pathovar *vesicatoria* (Xcv) and resistance genes (*Bs1*, *Bs2*, *Bs3*) that determine resistance to particular races of *Xcv* expressing the avirulence genes have been studied (Herbers et al. 1992). The protein product of the avirulence gene has repeat units that determine the race specificity. As new deletions of repeat units occur in the *avr* gene of the pathogen, new resistance genes were unmasked (Herbers et al. 1992). The frequency of mutations in *avr* genes affects the race composition of the pathogen and the durability of the corresponding plant resistance (Stall et al. 2009). Multigenic resistance has been shown to be more durable than hypersensitive resistance controlled by single genes (Stall et al. 2009).

Phytophthora capsici, an oomycetes whose spores survive for years in the soil can be spread via water and cause root rot, crown rot, fruit rot, and foliar blight. Several resistant varieties of peppers are available. Polygenic nature of resistance to *P. capsici* and QTLs has been identified. Molecular biology research over the past few decades have identified the nature of disease resistance genes. Nucleotide-binding site leucine-rich repeat proteins (NBS-LRR), and receptor-like proteins (RLPs) have been identified as resistance genes in many different crops (Jones and Takemoto 2004). Using the genome sequence data and information on the QTLs on chromosome 5, Kim et al. (2019) developed markers based on clustered resistance gene analogs to identify plants with *P. capsici* resistance. Transcriptomic studies comparing the resistant variety CM334 with a sensitive variety identified that many genes were repressed upon inoculation with *P. capsici* in the sensitive variety and 22 genes uniquely expressed in the resistant line are likely the candidate genes for resistance (Richins et al. 2010).

Compared to disease resistance traits, the genetics of resistance to other pests has not been well explored. Studies on thrip resistance identified QTLs for this trait in specific mapping populations (Maharijaya et al. 2015) and leaf position and ontogeny

affect leaf resistance to thrips (Visschers et al. 2019). More recently, metabolomic research showed that diterpenes and flavonoids may have a role in resistance to thrips (Maharijaya et al. 2019).

5.2.8 Root-Knot Nematodes

Soil-borne plant-parasitic nematodes are major pathogens limiting pepper productivity. Root-knot nematodes (*Meloidogyne* spp.) are major pathogens for the Solanaceous crops. *M. arenaria*, *M. incognita*, *M. javanica*, *M. enterolobii,* and *M. chitwoodi* are distributed in the tropics and *M. hapla* in temperate regions (Jones et al. 2013). Juveniles (J2) hatched from the eggs penetrate the roots, migrate inside the root cells to form permanent feeding sites with giant cells. J2 swells and molts three times to reach the adult stage. While adult males leave the root, the adult female enlarges to become pear-shaped and lays eggs encased in gelatinous matrix. Root-knot nematode infection causes galling of the roots, weakens the plants, resulting in varying degrees of yield losses.

Pre-plant soil sterilization using nematicidal chemicals is the most effective way to control root-knot nematodes in pepper production. Among the many products, fumigants have shown most effectiveness. However, fumigation chemicals are damaging to the environment. The often-used methyl bromide was banned by regulators, because of its high ozone depletion potential. Researchers have now recognized the need for an integrated approach to manage crops against root-knot nematodes (Seid et al. 2015). Use of soil solarization, flooding, and fallow treatment of the soil; organic amendments with nematicidal activity; biological controls; and the use of root-knot nematode-resistant varieties have been tested in many experimental studies with varying degrees of success. Nematophagous egg-parasitising *Purpureocillium lilacinum*, *Pochonia chlamydosporia*, *Trichoderma* spp., *Aspergillus* spp., *Verticillium chlamydosporium*, obligate parasite *Pasteuria penetrans*, rhizobacteria such as *Paenibacillus polymyxa* (Khan et al. 2008), and *Pseudomonas* spp. have been shown to be effective for the biocontrol of root-knot nematodes (Li et al. 2015b). When cultural methods were used to suppress root-knot nematodes, the effectiveness depended on multiple factors. Therefore, more research is needed to find the best methods of their applications to develop reproducible suppression of root-knot nematodes under field conditions.

Use of root-knot nematode-resistant varieties is another interesting approach requiring little input during the growing season and is environmentally sound (Fuller et al. 2008). Sarath Babu et al.'s (2011) review on pepper genetic resources against arthropods, nematodes, and pathogen has listed more than 40 accessions reportedly tolerant or highly resistant to different species of root-knot nematodes. Root-knot nematode resistance in the Solanaceae is mainly dominant, controlled by few major genes. Nine different dominant genes have been named for root-knot nematode resistance in different populations of peppers. Out of these, *N*, *Me1* and *Me3* (= *Me7*) were evaluated for resistance to *M. incognita*, *M. javanica*, *M. arenaria,* and *M. haplanaria*

(Hajihassani et al. 2019). One recessive gene was suggested in a variety "Carolina Wonder", associated with the dominant R gene named N (Thies and Fery 2000). The root-knot nematode-resistant gene *Me* is clustered with other pathogen resistance genes (for potyviruses and bacterial leaf spot) on chromosome P9 (Djian-Caporalino et al. 2007). *C. annuum* variety "CM334" which is the source of resistance to *Phytophthora* spp. and potyvirus has been shown to be resistant to three most damaging *Meloidogyne* spp. and it was suggested that accumulation of chlorogenic acid was suggested to be linked to resistance (Pegard et al. 2005). It has been proposed that activities of transposable elements and gene duplications followed by "neofunctionalization" has led to the evolution of multiple pathogen resistance genes in pepper (Barbary et al. 2015).

Huang et al. (2006) used RNAi gene silencing method to engineer a double-stranded RNA for a root-knot nematode's parasitism gene (16D10) in a model plant to achieve resistance against four major root-knot nematode species. In the same year, Yadav et al. (2006) reported this strategy of using RNAi-based silencing of essential genes in the nematode to achieve root-knot nematode resistance in a model species. Based on the success of these initial studies, others have now generated root-knot nematode-resistant transgenic tomato (Dutta et al. 2015; Zhuo et al. 2017) and eggplant (Sivakumara et al. 2017). There is great potential to apply the RNAi technology in *Capsicum* also as the nematode genes identified in these studies will also be effective for achieving root-knot nematode resistance in peppers.

5.2.9 Genetic Maps and Comparative Genomics

There are multiple reports on the development of genetic maps for *Capsicum* sp. Different types of markers were used to build linkage maps so that chromosomal regions controlling specific quantitative traits could be identified using conventional biparental mapping populations. The availability of genetic maps in tomato was helpful. For example, Livingstone et al. (1999) developed markers by hybridizing with tomato probes. Wu et al. (2009) mapped 587 orthologous markers in pepper and showed that tomato and pepper shared 35 conserved syntenic segments with which gene/marker order is well preserved. Multiple linkage maps were reported using RAPD markers (Rodriguez et al. 1999), RFLP and amplified fragment length polymorphism (AFLP) (Kang et al. 2001), function-related markers (Lefebvre et al. 2002), microsatellite loci (Lee et al. 2004), linkage maps with integrated markers (Paran et al. 2004), SSR markers (Minamiyama et al. 2006), and simple sequence repeat (SSR) markers based on expressed sequence tags (Yi et al. 2006). While these and many other developments are significant in QTL analyses for specific traits, the introduction of single nucleotide polymorphism (SNP) markers have opened multiple new opportunities for dense linkage maps with which to map QTL and identify candidate genes for important quantitative traits.

Transcriptome sequences, genotyping-by-sequencing, and whole-genome sequencing methods have accelerated the availability of large number of DNA-based

SNP markers (Ashrafi et al. 2012; Kim et al. 2017; Lu et al. 2012; Hulse-Kemp et al. 2018). Lee et al. (2013) developed 145 primer pairs for high-resolution melting (HRM) markers based on NGS resequencing. Others have identified indels (1–5 bp) in two pepper inbred lines and validated and mapped 252 InDel markers (Li et al. 2015a). Rehrig et al. (2014) built a high-density map with 3887 markers in a set of recombinant inbred lines (RILs) derived from a *Phytophthora capsici* resistant CM334 and sensitive parent Early Jalapeno. This study led to the identification of multiple QTL for resistance and one candidate gene for a major QTL on chromosome 5. The gene was identified as CaDMR1, coding for a homoserine kinase (Rehrig et al. 2014). Mahasuk et al. (2016) identified three major QTL for anthracnose disease resistance using two SNP maps. Ahn et al. (2018) sequenced *C. baccatum* and *C. annuum* accessions to identify 4.8 million polymorphic SNP loci and developed 306,871 high-resolution melting (HRM) marker primer sets. This study identified thousands of SNPs associated with disease resistance genes (Ahn et al. 2018). *Capsicum chinense*, unlike *C. annuum*, has multiple flowers per node and is flowering later. Zhu et al. (2019) analyzed a F_2 population derived from these two species to identify QTLs for flowering time, a trait linked to yield and is sensitive to climate change. Together these studies illustrate that marker-assisted selection strategies can be applied in pepper breeding programs to best utilize the available genetic variation.

5.2.10 Genome-Wide Association Mapping

Genome-wide association mapping is an alternate new approach to using biparental mapping populations, to identify QTLs for complex traits. For example, in a study of 473 accessions of the model plant *Arabidopsis thaliana*, by using >200 thousand SNP markers, Li et al. (2010) identified 12 major QTLs for flowering time a trait sensitive to climate with pleiotropic effects on yield. Nimmakayala et al. (2016a) is the first to report the use of GWAS to map traits in a diverse collection of *C. annuum*. Their study revealed that SNPs in genes encoding ankyrin-like protein, IK13 family protein, ABC transporter G family, and pentatricopeptide repeat protein are the major markers for capsaicinoids (Nimmakayala et al. 2016a). Nimmakayala et al. (2016b)'s GWAS study reported several significantly associated SNPs for genes controlling peduncle length.

DNA variation has also been used for developing resources to study genetic diversity in populations of plants, discovery of SNP markers, and evaluation of gene expression. Hill et al. (2013) developed a 30 K Unigene Pepper GeneChip, an important community resource.

5.2.11 Whole Genome Sequencing

In 2014, the first whole-genome sequencing of cultivated and wild peppers was achieved (Qin et al. 2014). Efforts by two teams led to the availability of genome sequences for *C. annuum* cultivars CM334, Zunla-1 and semiwild progenitor *C. annuum* var. *glabrisculum* and resequencing of two cultivars Perennial and Dempsey (Qin et al. 2014, Kim et al. 2014). Kim et al. (2014) reported the genome sequence for *C. chinense* also. Pepper genome is much larger than those of other members of the Solanaceous crops for which genome sequences are available: its genome size of 3480 Mb is several-fold larger than that of potato (840 Mb), tomato (950 Mb) and eggplant (1127 Mb). It was determined that 76–81% of the pepper genome was composed of transposable elements, primarily long terminal repeat elements of the *Gypsy* clade (Qin et al. 2014). Although the functional significance of large expansion of the heterochromatin region of the genome is not known, pepper genome is an ideal model to study the phenomenon (Park et al. 2011).

Genome sequence data made it possible to infer important conclusions on the specific traits altered during domestication of this crop (Qin et al. 2014). Genes identified in regions of the genome with signatures of artificial selection included those related to transcriptional regulation, defense, stress, seed dormancy, and fruit development (Qin et al. 2014). While homologs of genes potentially involved in capsaicinoid biosynthesis were found in Arabidopsis, tomato, and potato, pepper had specific duplications in 13 gene families suggesting gene duplication and neofunctionalization. Analysis of At3 (Pun1) gene, a gene coding for a putative acyltransferase known to control capsaicin synthesis, indicated that there were three copies of this gene duplicated in tandem in the pungent accession while there was deletion in the putative promoter region of the gene in the non-pungent accession (Qin et al. 2014). Ou et al. (2018) compared next-generation sequencing (NGS) data of 355 *C. annuum*, four *C. baccatum*, 11 *C. chinense*, and 13 *C. frutescens* cultivars and analyzed for gene presence-absence variation. The resulting pepper pan-genome browser (http://www.pepperpan.org:8012/) will be an in important resource for *Capsicum* geneticists.

5.3 Transgenic Technologies and Genome Editing

Tissue culture regeneration of plants from cultured tissues of *Capsicum* is somewhat difficult compared to other members of the Solanaceae such as tobacco and tomato (Gammoudi et al. 2018; Kothari et al. 2010). Microspore embryogenesis a method useful for producing doubled haploids in breeding has been optimized (Heidari-Zefreh et al. 2019). Successful procedures exist for expressing foreign genes in transgenic pepper plants using direct gene transfer methods or agrobacterium-mediated gene transfer methods (Ko et al. 2007; Manoj Kumar et al. 2011; Ortega et al. 2018).

Cucumber mosaic virus, a member of the Cucumovirus, has a broad host range, affects peppers, and is vectored by aphids. Introduction of cucumber mosaic virus

coat protein (Zhu et al. 1996) or satellite RNA (Kim et al. 1997) resulted in cucumber mosaic virus-resistant plants. Similar strategy was used to make virus-resistant transgenic peppers by expressing coat proteins from both cucumber mosaic virus and tomato mosaic virus (Shin et al. 2002). Song and Ryu (2017) devised a strategy to silence genes involved in the defense system to stop the viral infection using RNAi, where double-stranded RNA is cleaved by Dicer. Genome editing using CRISPR/Cas9 system to introduce mutations in specific target genes has not been achieved in Capsicum, although the potential of this technology will soon be realized (Van Eck 2018). As more candidate genes are identified for horticulturally relevant traits via genomic tools in different species, we can expect that the use of gene transfer and genome editing for the purpose of breeding improved varieties will increase in the future.

Acknowledgements The author thanks Jingwei Fu (University of Florida) for help with figures. Funding for the author's research on Capsicum breeding is gratefully acknowledged: grants were from the College of Agriculture and Life Sciences, the US Department of Agriculture's Agricultural and Marketing Service, and the Florida Department of Agriculture and Consumer Services.

References

Abdel-Aal EM, Akhtar H, Zaheer K, Ali R (2013) Dietary sources of lutein and zeaxanthin carotenoids and their role in eye health. Nutrients 5:1169–1185

Abraham-Juarez MD, Rocha-Granados MD, Lopez MG, Rivera-Bustamante RF, Ochoa-Alejo N (2008) Virus-induced silencing of *Comt*, *pAmt* and *Kas* genes results in a reduction of capsaicinoid accumulation in chili pepper fruits. Planta 227:681–695

Ahn S, Badenes-Perez FR, Heckel DG (2011) A host-plant specialist, *Helicoverpa assulta*, is more tolerant to capsaicin from *Capsicum annuum* than other noctuid species. J Insect Physiol 57:1212–1219

Ahn Y, Manivannan A, Karna S, Jun T, Yang E, Choi S, Kim J, Kim D, Lee E (2018) Whole genome resequencing of *Capsicum baccatum* and *Capsicum annuum* to discover single nucleotide polymorphism related to powdery mildew resistance. Sci Rep 8:5188

Airaki M, Leterrier M, Mateos RM, Valderrama R, Chaki M, Barroso JB, Rio LA, Palma JM, Corpas FI (2012) Metabolism of reactive oxygen species and reactive nitrogen species in pepper (*Capsicum annuum* L.) plants under low temperature stress. Plant, Cell Environ 35:281–295

Aktas H, Abak K, Cakmak I (2006) Genotypic variation in the response of pepper to salinity. Sci Hort 110:260–266

Aktas H, Abak K, Eker S (2012) Anti-oxidative responses of salt-tolerant and salt-sensitive pepper (*Capsicum annuum* L.) genotypes grown under salt stress. J HortSci Biotechnol 87:360–366

Ali AM, Kelly WC (1992) The effects of interfruit competition on the size of sweet pepper (*Capsicum annuum* L.) fruits. Sci Hort 52:69–76

Aloni B, Karni L, Rylski I, Cohen Y, Lee Y, Fuchs M, Moreshet S, Yao C (1998) Cuticular cracking in pepper fruit. I. Effects of night temperature and humidity. J Hort Sci Biotechnol 73:743–749

Aloni B, Peet M, Pharr M, Karni L (2001) The effect of high temperature and high atmospheric CO2 on carbohydrate changes in bell pepper (*Capsicum annuum*) pollen in relation to its germination. Physiol Plant 112:505–512

Antonio AS, Wiedemann LSM, Veiga VF Jr (2018) The genus *Capsicum*: a phytochemical review of bioactive secondary metabolites. RSC Advances 8:25767–25784

Arce-Rodriguez M, Ochoa-Alejo N (2017) An R2R3-MYB transcription factor regulates capsaicinoid biosynthesis. Plant Physiol 174:1359–1370

Ashrafi H, Hill T, Stoffel K, Kozik A, Yao JQ, Chin-Wo SR, Van Deynze A (2012) *De Novo* assembly of the pepper transcriptome (*Capsicum annuum*): a benchmark for *in silico* discovery of SNPs. SSRs and candidate genes. BMC Genomics 13:571

Azuma R, Ito N, Nakayama N, Suwa R, Nguyen TN, Larringa-Mayoral JA, Esaka M, Fujiyama H, Saneoka H (2010) Fruits are more sensitive to salinity than leaves and stems in pepper plants (*Capsicum annuum* L.). Sci Hort 125:171–178

Barbary A, Djian-Caporalino C, Palloix A, Castagnone-Sereno (2015) Host genetic resistance to root-knot nematodes, Meloidogyne spp., in Solanaceae: from genes to the field. Pest Manag Sci 71:1591–1598

Baas-Espinola FM, Castro-Concha LA, Vazquez-Flota FA, Miranda-Ham ML (2016) Capsaicin synthesis requires *in situ* phenylalanine and valine formation in in vitro maintained placentas from Capsicum chinense. Molecules 21:799

Barchi L, Lefebvre V, Sage-Palloix A, Lanteri S, Palloix A (2009) QTL analysis of plant development and fruit traits in pepper and performance of selective phenotyping. Theor Appl Genet 118:1157–1171

Barchenger DW, Clark RA, Gniffe PA, Ledesma DR, Lin S, Hanson P, Kumar S (2018) Stability of yield and yield components of pepper (*Capsicum annuum*) and evaluation of publicly available predictive meteorological data in East and Southeast Asia. HortScience 53:1776–1783

Batista-Silva W, Nascimento VL, Medeiros DB, Nunes-Nesi A, Ribeiro DM, Zsogon A, Araujo WL (2018) Modifications in organic acid profiles during fruit development and ripening: correlation or causation? Front Plant Sci 9:1689

Belakbir A, Ruiz JM, Romero L (1998) Yield and fruit quality of pepper (*Capsicum annuum* L.) in response to bioregulators. HortScience 33:85–87

Ben-Chaim A, Paran I (2000) Genetic analysis of quantitative traits in pepper (*Capsicum annuum*). J Amer Soc Hort Sci 125:66–70

Ben-Chaim A, Paran I, Grube RC, Jahn M, Van Wijk R, Peleman J (2001) QTL mapping of fruit-related traits in pepper (*Capsicum annuum*). Theor Appl Genet 102:1016–1028

Ben-Chaim A, Borovsky Y, De Jong W, Paran I (2003) Linkage of the A locus for the presence of anthocyanin and fs10.1, a major fruit-shape QTL in pepper. Theor Appl Genet 106:889–894

Ben-Chaim A, Borovsky Y, Falise M, Mazourek M, Kang BC, Paran I, Jahn M (2006) QTL analysis for capsaicinoid content in *Capsicum*. Theor Appl Genet 113:1481–1490

Ben-Chaim A, Borovsky Y, Rao G, Zamir D, Paran I (2013) Comparative QTL mapping of fruit size and shape in tomato and pepper. Isr J Plant Sci 54:191–203

Bhutia ND, Seth T, Shende VD, Dutta S, Chattopadhyay A (2015) Estimation of heterosis, dominance effect and genetic control of fresh fruit yield, quality and leaf curl disease severity traits of pepper (*Capsicum annuum* L.) Sci Hortic 182:47–55

Blum E, Mazourek M, O'Connell M, Curry J, Thorup T, Liu K, Jahn M, Paran I (2003) Molecular mapping of capsaicinoid biosynthesis genes and quantitative trait loci analysis for capsaicinoid content in *Capsicum*. Theor and Appl Gen 108:79–86

Bojorquez-Quintal E, Ruiz-Lau N, Velarde-Buendia A, Echevarria-Machado I, Pottosin I, Martinez-Esteves M (2016) Natural variation in primary root growth and K + retention in roots of habanero pepper (*Capsicum chinense*) under salt stress. Funct Plant Biol 43:1114–1125

Boswell VR (1937) Improvement and genetics of tomatoes, peppers, eggplant. United States Government Printing Office, Yearbook of Agriculture, Washington, pp 176–206

Canto-Flick A, Balam-Uc E, Bello-Bello JJ, Lecona-Guzman C, Solis-Marroquin D, Aviles-Vinas S, Gomez-Uc E, Lopez-Puc G, Santana-Buzzy N, Iglesias-Andreu LG (2008) Capsaicinoids content in habanero pepper (*Capsicum chinense* Jacq.): hottest known cultivars. HortScience 43:1344–1349

Carrizo Garcia C, Barfuss MH, Sehr EM, Barboza GE, Samuel R, Moscone EA, Ehrendorfer F (2016) Phylogenetic relationships, diversification and expansion of chili peppers (*Capsicum*, Solanaceae). Ann Bot 118:35–51

Chartzoulakis K, Klapaki G (2000) Response of two greenhouse pepper hybrids to NaCl salinity during different growth stages. Sci Hortic 86:247–260
Chen HC, Klein A, Xiang M, Backhaus RA, Kuntz M (1998) Drought- and wound-induced expression in leaves of a gene encoding a chromoplast carotenoid-associated protein. Plant J 14:317–326
Cheng J, Shen H, Yang X, Yu S, Sun Z, Sun X (2008) Changes in biochemical characteristics related to firmness during fruit development of pepper (*Capsicum annuum* L.). Eur J Hort Sci 5:155–161
Cheng Q, Li T, Ai Y, Lu Q, Wang Y, Sun L, Shen H (2019) Complementary transcriptomic and proteomic analysis reveals a complex network regulating pollen abortion in GMS (*msc-1*) pepper (*Capsicum annuum* L.). Intl J Mol Sci 20:1789
Crosby KM (2007) Pepper. In: Prohens-Tomas J, Nuez F (eds) Vegetables II. New York, Springer, pp 221–247
Cruz-Huerta N, Williamson JG, Darnell RL (2011) Low night temperature increases ovary size in sweet pepper cultivars. HortScience 46:396–401
Deshpande RB (1935) Studies in Indian chillies. 4. Inheritance of pungency in *Capsicum annuum*. L. Indian J Agri Sci 5:513–516
Dhall RK, Cheema DS (2010) Use of male sterility in hybrid seed production in chilli (*Capsicum annuum* L.): a review. J Res Punjab Agri Univ 47:46–52
Dijian-Caporalino C, Fazari A, Arguel MJ, Vernie T, VandeCasteele C, Faure I et al (2007) Root-knot nematode (*Meloidogyne* spp.) *Me* resistance genes in pepper (*Capsicum annuum* L.) are clustered on the P9 chromosome. Theor Appl Genet 114:473–486
Do HM, Lee SC, Jung HW, Sohn KH, Hwang BK (2004) Differential expression and *in situ* localization of a pepper defensing (CaDEF1) gene in response to pathogen infection, abiotic elicitors and environmental stresses in *Capsicum annuum*. Plant Sci 166:1297–1305
Dutta TK, Papolu PK, Banakar P, Choudhary D, Sirohi A, Rao U (2015) Tomato transgenic plants expressing hairpin construct of a nematode protease gene conferred enhanced resistance to root-knot nematodes. Front Microbiol 6:260
Eggink PM, Maliepaard C, Tikunov Y, Haanstra JPW, Bovy AG, Visser RGF (2012) A taste of sweet pepper: volatile and non-volatile chemical composition of fresh sweet pepper (*Capsicum annuum*) in relation to sensory evaluation of taste. Food Chem 132:302–310
Elibox W, Meynard CP, Umaharan P (2017) Fruit volume and width at harvest can be used to predict shelf life in pepper (*Capsicum chinense* Jacq.). Trop Agri (Trinidad) 94:122–131
Erickson AN, Markhart AH (2001) Flower production, fruit set, and physiology of bell pepper during elevated temperature and vapor pressure deficit. J Am Soc Hortic Sci 126:697–702
Erickson AN, Markhart AH (2002) Flower developmental stage and organ sensitivity of bell pepper (*Capsicum annuum* L.) to elevated temperature. Plant, Cell Environ 25:123–130
FAO (2017) www.fao.org/statistics/en. Accessed 1 Jun 2019
Fuller VL, Lilley CJ, Urwin PE (2008) Nematode resistance. New Phytol 180:27–44
Gammoudi N, PedroT San, Ferchichi A, Gisbert C (2018) Improvement of regeneration in pepper: a recalcitrant species. Vitro Cell Dev Biol Plant 54:145–153
Geleta LF, Labuschangne MT, Viljoen CD (2004) Relationship between heterosis and genetic distance based on morphological traits and AFLP markers in pepper. Plant Breed 123:467–473
Govindarajan VS, Salzer UJ (1985) Capsicum-production, technology, and quality. Part 1: History, botany, cultivation, and primary processing. Crit Rev Food Sci Nutr 22:109–176
Guan DY, Yang F, Xia XQ, Shi YY, Yang S, Cheng W, He SL (2018) CaHSL1 acts as a positive regulator of pepper thermotolerance under high humidity and is transcriptionally modulated by CaWRKY40. Front Plant Sci 9:1802
Guiffrida F, Cassaniti C, Leonardi C (2013) The influence of rootstock on growth and ion concentrations in pepper (*Capsicum annuum* L.) under saline conditions. J Hort Sci Biotechnol 88:110–116
Guo M, Liu JH, Lu JP, Zhai YF, Wang H, Gong ZH, Wang SB, Lu MH (2015) Genome-wide analysis of the CaHsp20 gene family in pepper: comprehensive sequence and expression profile analysis under heat stress. Front Plant Sci 6:806

Guo M, Liu JH, Ma X, Zhai YF, Gong ZH, Lu MH (2016) Genome-wide analysis of the Hsp70 family genes in pepper (*Capsicum annuum* L.) and functional identification of CaHsp70-2 involvement in heat stress. Plant Sci 252:246–256

Guzman I, Hamby S, Romero J, Bosland PW, O'Connell MA (2010) Variability of carotenoid biosynthesis in orange colored *Capsicum* spp. Plant Sci 179:49–59

Ha S, Kim J, Park J, Lee S, Cho K (2007) A comparison of the carotenoid accumulation in Capsicum varieties that show different ripening colours: deletion of the capsanthin-capsorubin synthase gene is not a prerequisite for the formation of a yellow pepper. J Exp Bot 58:3135–3144

Hajihassani A, Rutter WB, Luo X (2019) Resistant pepper carrying *N*, *Me1*, and *Me3* have different effects on penetration and reproduction of four major *Meloidogyne* species. J Nematol. https://doi.org/10.21307/jofnern-2019-020

Han K, Lee HY, Ro N, Hur O, Lee J, Kwon J, Kang BC (2018) QTL mapping and GWAS reveal candidate genes controlling capsaicinoid content in *Capsicum*. Plant Biotechnol J 16:1546–1558

Heidari-Zefreh AA, Shariatpanahi ME, Mousavi A, Kalatejari S (2019) Enhancement of microspore embryogenesis induction and plantlet regeneration of sweet pepper (*Capsicum annuum* L.) using putrescine and ascorbic acid. Protoplasma 256:13–24

Herbers K, Conrads-Strauch J, Bonas U (1992) Race-specificity of plant resistance to bacterial spot disease determined by repetitive motifs in a bacterial avirulence protein. Nature 356:172–174

Heuvelink E, Korner O (2001) Parthenocarpic fruit growth reduces yield fluctuation and blossom-end rot in sweet pepper. Ann Bot 88:69–74

Hill TA, Ashrafi H, Reyes-Chin-Wo S, Yao JQ, Stoffel K, Truco MJ, Kozik A, Michelmore RW, Van Deynze A (2013) Characterization of *Capsicum annuum* genetic diversity and population structure based on parallel polymorphism discovery with a 30 K unigene pepper genechip. PLoS ONE 8:e56200

Hornero-MendezD., Guevara R, Minguez-Mosquera IM (2000) Carotenoid biosynthesis changes in five red pepper (*Capsicum annuum* L.) cultivars during ripening. Cultivar selection for breeding. J Agri Food Chem 48: 3857–3864

Huang G, Allen R, Davis EL, Baum TJ, Hussey RS (2006) Engineering broad root-knot resistance in transgenic plants by RNAi silencing of a conserved and essential root-knot nematode parasitism gene. Proc Natl Acad Sci USA 103:14302–14306

Huberman M, Riov J, Aloni B, Goren R (1997) Role of ethylene biosynthesis and auxin content and transport in high temperature-induced abscission of pepper reproductive organs. J Plant Growth Regul 16:129–135

Hulse-Kemp A, Maheshwari S., Stoffel K, Hill TA, Jaffe D, Williams SR, Weisenfeld N, Ramakrishnan S, Kumar V, Shah P, Schatz MC, Church DM, Van Deynze A (2018) Reference quality assembly of the 3.5-Gb genome of *Capsicum annuum* from a single linked-read library. Hort Res 5:4

Hurtado-Hernandez H, Smith PG (1985) Inheritance of mature fruit color in *Capsicum annuum* L. J Hered 76:211–213

IPPC (2014) Climate change 2014: synthesis report. Contribution of working Groups I, II and III to the fifth assessment report of the intergovernmental panel on climate change. [Core Writing Team, Pachauri RK, Meyer LA (eds)]. Geneva, Switzerland, IPCC, 151 pp. https://www.ipcc.ch/report/ar5/syr/

Isbat M, Zeba N, Kim SR, Hong CB (2009) A BAX inhibitor-1 gene in *Capsicum annuum* is induced under various abiotic stresses and endows multi-tolerance in transgenic tobacco. J Plant Physiol 166:1685–1693

Jang YK, Jung ES, Lee H, Choi D, Lee CH (2015) Metabolomic characterization of hot pepper (*Capsicum annuum* "CM334") during fruit development. J Agri Food Chem 63:9452–9460

Jarret RL, Barboza GE, Batista FRC, Berke T, Chou Y, Hulse-Kemp A, Ochoa-Alejo N, Tripodi P, Veres A, Garcia CC, Csillery G, Huan Y, Kiss E, Kovacs Z, Kondrak M, Arce-Rodriguez ML, Scaldaferro MA, Szoke A (2019) Capsicum—An abbreviated compendium. J Amer Soc Hort Sci 144:3–22

Jo YD, Ha Y, Lee JH, Park M, Bergsma AC, Choi HI, Goritschnig S, Kloosterman B, van Dijk PJ, Choi D, Kang BC (2016) Fine mapping of Restorer-of-fertility in pepper (*Capsicum annuum* L.) identified a candidate gene encoding a pentatricopeptide repeat (PPR)-containing protein. Theor Appl Genet 129: 2003-2017

Jones JT, Haegeman A, Danchin EGJ, Gaur HS, Helder J, Jones MGK, Kikuchi T, Manzanilla-Lopez R, Palomares-Rius JE, Wesemael WML, Perry RN (2013) Top 10 plant-parasitic nematodes in molecular plant pathology. Mol Plant Pathol 14:946–961

Jones DA, Takemoto D (2004) Plant innate immunity—direct and indirect recognition of general and specific pathogen-associated molecules. Curr Opin Immunol 16:48–62

Kaiser S (1935) The factors governing shape and size in Capsicum fruits; a genetic and developmental analysis. Bull Torr Bot Club 62:433–445

Kang BC, Nahm SH, Huh JH, Yoo HS, Yu JW, Lee MH, Kim BD (2001) An interspecific (*Capsicum annuum* × *C. chinense*) F-2 linkage map in pepper using RFLP and AFLP. Theor Appl Genet 102:531–539

Keyhaninejad N, Curry J, Romero J, O'Connell MA (2014) Fruit specific variability in capsaicinoid accumulation and transcription of structural and regulatory genes in *Capsicum* fruit. Plant Sci 215:59–68

Khan Z, Kim SG, Jeon YH, Khan HU, Son SH, Kim YH (2008) A plant growth promoting rhizobacterium *Paenibacillus polymyxa* strain GBR-1, suppresses root-knot nematode. Bioresour Technol 99:3016–3023

Kilcrease J, Rodriguez-Uribe L, Richins RD, Victorino AJM, O'Connell MA (2015) Correlations of carotenoid content and transcript abundance for fibrillin and carotonogenic enzymes in *Capsicum annum* fruit pericarp. Plant Sci 232:57–66

Kim DS, Hwang BK (2014) An important role of the pepper phenylalanine ammonia-lyase gene (PAL1) in salicylic acid-dependent signaling of the defense response to microbial pathogens. J Exp Bot 65:2295–2306

Kim DS, Kim DH, Yoo JH, Kim BD (2006) Cleaved amplified polymorphic sequence and amplified fragment length polymorphism markers linked to the fertility restorer gene in chili pepper (*Capsicum annuum* L.). Mol Cells 21:135–140

Kim DH, Kang JGK, Kim BD (2007) Isolation and characterization of the cytoplasmic male sterility-associated orf456 gene of chili pepper (*Capsicum annuum* L.) Plant Mol Biol 63: 519–532

Kim S, Park M, Yeom SI, Kim YM Lee JM, Lee, HA, Seo E, Choi J, Cheong K, Kim KT, Jung K, Lee GW, Oh SK, Bae C, Kim SB, Lee, HY, Kim, SY, Kim, MS, Kang BS, Jo YD, Yang HB, Jeong HJ, Kang WH, Kwon JK, Shin C, Lim JY, Park JH, Huh JS, Kim BD, Kim O, Cohen I, Paran MC, Suh SB, Lee YK, Kim Y, Shin SJ, Noh J, Park YS, Seo SY, Kwon HA, Kim JM, Park HJ, Kim SB, Choi PW, Bosland G, Reeves SH, Jo BW, Lee HT, Cho HS, Choi MS, Lee Y, YuY, Do Choi BS, Park A, van Deynze H, Ashrafi T, Hill WT, Kim HS, Pai HK, Ahn I, Yeam, JJ, Giovannoni JK, Rose I, Sørensen SJ, Lee RW, Kim IY, Choi BS, Choi JS, Lim YH, Lee, Choi, D. (2014) Genome sequence of the hot pepper provides insights into the evolution of pungency in Capsicum species. Nat Genet 46:270–278

Kim S, Park J, Yeom S, Kim YM, Seo E, Kim K, Kim M, Lee J, Cheong K, Shin H, Kim S, Han K, Lee J, Park M, Lee H, Lee H, Lee Y, Oh S, Lee JH, Choi E, Choi E, Lee S, Jeon J, Kim H, Choi G, Song H, Lee J, Lee S, Kwon J, Lee H, Koo N, Hong Y, Kim RW, Kang WH, Huh JH, Kang BC, Yang T, Lee YH, Bennetzen JL, Choi D (2017) New reference genome sequences of hot pepper reveal the massive evolution of plant disease resistance genes by retroduplication. Genome Biol 18:210

Kim N, Kang WH, Lee J, Yeom S (2019) Development of clustered resistance gen analogs-based markers of resistance to *Phytophthora capsici* in chilli pepper. Biomed Res Int 1093186

Kim SJ, Lee SJ, Kim B-D, Paek K-H (1997) Satellite-RNA-mediated resistance to cucumber mosaic virus in transgenic plants of hot pepper (*Capsicum annuum* cv. Golden Tower). Plant Cell Reports 16 (12):825–830

Kissinger M, Tuvia-Alkalai S, Shalom Y, Fallik E, Elkind Y, Jenks MA, Goodwin MS (2005) Characterization of physiological and biochemical factors associated with postharvest water loss in ripe pepper fruit during storage. J Am SocHortic Sci 130:735–741

Kothari SL, Joshi A, Kachhwaha S, Ochoa-Alejo N (2010) Chilli peppers—A review on tissue culture and trasngenesis. Biotechnol Adv 28:35–48

Ko MK, Soh H, Kim KM, Kim YS (2007) Stable production of transgenic pepper plants mediated by *Agrobacterium tumefaciens*. HortScience 42:1425–1430

Kraft KH, Brown CH, Nabhan GP, Luedeling E, Ruiz JJL, Coppens d'Eeckenbrugge G, Hijmans RJ, Gepts P (2013) Multiple lines of evidence for the origin of domesticated chili pepper, *Capsicum annuum*, in Mexico. Proc Natl Acad Sci USA 111:6165–6170

Lee SJ, Kim BS (2012) Evaluation of pepper genetic resources (*Capsicum* spp.) for disease resistance breeding. Kor J Hort Sci Technol 30:185–191

Lee JM, Nahm SH, Kim YM, Kim BD (2004) Characterization and molecular genetic mapping of microsatellite loci in pepper. Theor Appl Genet 108:619–627

Lee J, Yoon JB, Park HG (2008) A CAPS marker associated with the partial restoration of cytoplasmic male sterility in chili pepper (*Capsicum annuum* L.). Mol Breed 21:95–104

Lee J, Park S, Do J, Han JH, Choi D, Yoon JB (2013) Development of a genetic map of chili pepper using single nucleotide polymorphism markers generated from next generation resequencing of parents. Kor J Hort Sci Technol 31:473–482

Lefebvre V, Pflieger S, Thabuis A, Caranta C, Blattes A, Chauvet JC, Daubeze AM, Palloix A (2002) Towards the saturation of the pepper linkage map by alignment of three intraspecific maps including known-function genes. Genome 45:839–854

Li Y, Huang Y, Bergelson J, Nordborg M, Borevitz JO (2010) Association mapping of local climate-sensitive quantitative trait loci in *Arabidopsis thaliana*. Proc Natl Acad Sci USA 107:21199–21204

Li WP, Cheng JW, Wu ZM, Qin C, Tan S, Tang X, Cui JJ, Zhang L, Hu KL (2015a) An InDel-based linkage map of hot pepper (*Capsicum annuum*). Mol Breed 35:32

Lightbourn GJ, Stommel JR, Griesbach RJ (2007) Epistatic interactions influencing anthocyanin gene expression in *Capsicum annuum*. J Am Soc Hort Sci 132:824–829

Liu Y, Tikunov Y, Schouten RE, Marcelis LFM, Visser RGF, Bovy A (2018) Anthocyanin biosynthesis and degradation mechanisms in Solanaceous vegetables: a review. Front Chem 6:52

Locascio SJ, Stall WM (1994) Bell pepper yield as influenced by plant spacing and row arrangement. J Am Soc Hort Sci 119:899–902

Lopez-Marin J, Gonzalez A, Perez-Alfocea F, Egea-Gilabert C, Fernandez JA (2013) Grafting is an efficient alternative to shading screens to alleviate thermal stress in greenhouse-growth sweet pepper. Sci Hort 149:39–46

Lu F, Cho MC, Park YJ (2012a) Transcriptome profiling and molecular marker discovery in red pepper, *Capsicum annuum* L. TF68. Mol Biol Rep 39:3327–3335

Lu F, Kwon SW, Yoon MY, Kim KT, Cho MC, Yoon M, Park YJ (2012b) SNP marker integration and QTL analysis of 12 agronomic and morphological traits in F8 RILs of pepper (*Capsicum annuum* L.). Mol Cells 34:25–34

Li J, Zou C, Xu J, Ji X, Niu X, Yang J, Huang X, Zhang K (2015b) Molecular mechanisms of nematode-nematophagous microbe interactions: basis for biological control of plant-parasitic nematodes. Annu Rev Phytopathol 53:67–95

Lin W, Hill BD (2008) Neural network modeling to predict weekly yields of sweet peppers in a commercial greenhouse. Can J Plant Sci 88:531–536

Lin W, Dietmar F (2009) Combined analysis to characterize yield pattern of greenhouse-grown red sweet peppers. HortScience 44:362–365

Livingstone KD, Lackney VK, Blauth JR, van Wijk R, Jahn MK (1999) Genome mapping in *Capsicum* and the evolution of genome structure in the Solanaceae. Genetics 152:1183–1202

Maboko MM, Plooy D, Phillipus C (2015) Effect of plant growth regulators on growth, yield, and quality of sweet pepper plants grown hydroponically. HortScience 50:383–386

Mahasuk P, Struss D, Mongkolporn O (2016) QTLs for resistance to anthracnose identified in two Capsicum sources. Mol Breed 36:10

Maharijaya A, Vosman B, Pelgrom K, Wahyuni Y, De Vos RCH, Voorrips RE (2019) Genetic variation in phytochemicals in leaves of pepper (*Capsicum*) in relation to thrips resistance. Arthropod Plant Interact 13:1–9

Maharijaya A, Vosman B, Steenhuis-Broers G, Pelgrom K, Purwito A, Visser RGF, Voorrips RE (2015) QTL mapping of thrips resistance in pepper. Theor Appl Genet 128:1945–1956

Manoj Kumar A, Reddy KN, Manjulatha M, Arellano ES, Rohini S, Girija G (2011) A rapid, novel and high-throughput identification of putative bell pepper transformants generated through in planta transformation approach. Sci Hort 129:898–903

Mazourek M, Pujar A, Borovsky Y, Paran I, Mueller L, Jahn MM (2009) A dynamic interface for capsaicinoid systems biology. Plant Physiol 150:1806–1821

Meena OP, Dhaliwal MS, Jindal SK (2018) Development of cytoplasmic male sterile lines in chilli (*Capsicum annuum* L.) and their evaluation across multiple environments. Breed Sci 68:404–412

Mercado JA, Reid MS, Valpuesta V, Quesada MA (1997) Metabolic changes and susceptibility to chilling stress in *Capsicum annuum* plants grown at suboptimal temperature. Aust J Plant Physiol 24:759–767

Minamiyama Y, Tsuro M, Hirai M (2006) An SSR-based linkage map of *Capsicum annuum*. Mol Breed 18:57–169

Moury B, Selassie KG, Marchoux G, Daubeze AM, Palloix A (1998) High temperature effects on hypersensitive resistance to Tomato Spotted wilt Tospovirus (TSWV) in pepper (*Capsicum chinense* Jacq.). Eur J Plant Pathol 104:489–498

Naegele RP, Granke LL, Fry J, Hill TA, Ashrafi H, Van Deynze A, Hausbeck MK (2017) Disease resistance to multiple fungal and oomycete pathogens evaluated using a recombinant inbred line population in pepper. Phytopathology 107:1522–1531

Nascimento NF, Do Rego ER, Nascimento MF, Bruckner CH, Finger FL, Do Rego MM (2014) Combining ability for yield and fruit quality in the pepper *Capsicum annuum*. Genet Mol Res 13:3237–3249

Nimmakayala P, Abburi VL, Saminathan T, Alaparthi SB, Almeida A, Davenport B, Nadimi M, Davidson J, Tonapi K, Yadav L, Malkaram S, Vajja G, Hankins G, Harris R, Park M, Choi D, Stommel J, Reddy UK (2016a) Genome-wide diversity and association mapping for Capsaicinoids and fruit weight in *Capsicum annuum*. Sci Rep 6:38081

Nimmakayala P, Abburi VL, Saminathan T, Almeida A, Davenport B, Davidson J, Reddy CVCM, Hankins G, Ebert A, Choi D, Stommel J, Reddy UK (2016b) Genome-wide divergence and linkage disequilibrium analyses for *Capsicum baccatum* revealed by genome-anchored single nucleotide polymorphisms. Front Plant Sci 7:1646

Niu G, Rodriguez DS, Crosby K, Leskovar D, Jifon J (2010) Rapid screening for relative salt tolerance among chile pepper genotypes. HortScience 45:1192–1195

Ogawa D, Ishikawa K, Nunomura O, Mii M (2010) Correlation between fruit characters and degree of polysomaty in fruit tissues of *Capsicum*. J Jpn Soc Hort Sci 79:168–173

Oliveira Vilarinho LB, Silva DJH, Greene A, Salazar KD, Alves C, Eveleth M, Nichols B, Tehseen S, Khoury JK Jr, Johnson JV, Sargent SA, Rathinasabapathi B (2015) Inheritance of fruit traits in *Capsicum annuum*: heirloom cultivars as sources of quality parameters relating to pericarp shape, color, thickness and total soluble solids. J Am Soc Hort Sci 140:597–604

Ortega JL, Rajapakse W, Bagga S, Apodaca K, Lucero Y, Sengupta-Gopalan C (2018) An intragenic approach to confer glyphosate resistance in chile (*Capsicum annuum*) by introducing an in vitro mutagenized chile EPSPS gene encoding for a glyphosate resistant EPSPS protein. PLoS ONE 13:e0194666

Osorio S, Alba R, Nikoloski Z, Kochevenko A, Fernie AR, Giovannoni JJ (2012) Integrative comparative analyses of transcript and metabolite profiles from pepper and tomato ripening and development stages uncovers species-specific patterns of network regulatory behavior. Plant Physiol 159:1713–1729

Ou L, Li D, Lv J, Chen W, Zhang Z, Li X, Yang B, Zhou S, Yang S, Li W, Gao H, Zeng Q, Yu H, Ouyang B, Li F, Liu F, Zheng J, Liu Y, Wang J, Wang B, Dai X, Ma Y, Zou X (2018) Pan-genome of cultivated pepper (*Capsicum*) and its use in gene presence-absence variation analyses. New Phytol 220:360–363

Pabon-Mora N, Litt A (2011) Comparative anatomical and developmental analysis of dry and fleshy fruits of Solanaceae. Amer J Bot 98:1415–1436

Pagamas P, Nawata E (2008) Sensitive stages of fruit and seed development of chili pepper (*Capsicum annuum* L. var. Shishito) exposed to high-temperature stress. Sci Hortic 117:21–25

Palloix A, Ayme V, Moury B (2009) Durability of plant major resistance genes to pathogens depends on the genetic background, experimental evidence and consequences for breeding strategies. New Phytol 183:190–199

Paran I, Van der Voot JR, Lefebvre V, Jahn M, Landry L, Van Schriek M, Tanyolac B, Caranta C, Ben Chaim A, Livingstone K, Palloix A, Peleman J (2004) An integrated genetic linkage map of pepper (*Capsicum* spp.). Mol Breed 13:251–261

Paran I, Van der Knaap E (2007) Genetic and molecular regulation of fruit and plant domestication traits in tomato and pepper. J Exp Bot 58:3841–3852

Park M, Park J, Kim S, Kwon J, Park H, Bae IH, Yang T, Lee Y, Kang BC, Choi D (2011) Evolution of the large genome in *Capsicum annuum* occurred through accumulation of single-type LTR-retrotransposons and their derivatives. Plant J 69:1018–1029

Pegard A, Brizzard G, Fazari A, Soucaze O, Abad P, Djian-Caporalino C (2005) Histological characterization of resistance to different root-knot nematodes species related to phenolics accumulation in *Capsicum annuum*. Phytopathology 95:158–165

Penella C, Landi M, Guidi L, Nebauer SG, Pellegrini E, Bautista AS, Remorini D, Nali C, Lopez-Galarza S, Calatayud A (2016) Salt-tolerant rootstock increases yield of pepper under salinity through maintenance of photosynthetic performance and sinks strength. J Plant Physiol 193:1–11

Penella C, Nebauer SG, Lopez-Galarza S, Quinones A, Bautista AS, Calatayud A (2017) Grafting pepper onto tolerant rootstocks: an environmental-friendly technique to overcome water and salt stress. Sci Hort 226:33–41

Peterson PA (1958) Cytoplasmically inherited male sterility in Capsicum. Amer Natur 92:111–119

Pickersgill B (1997) Genetic resources and breeding of *Capsicum* spp. Euphytica 96:129–133

Pressman E, Moshkovitch H, Rosenfeld K, Shaked R, Gamliel B, Aloni B (1998) Influence of low night temperature on sweet pepper flower quality and the effect of repeated pollinations, with viable pollen, on fruit setting. J Hort Sci BioTechnol 73:131–136

Qin C, Yu C, Shen Y, Fang X, Chen L, Min UJ, Cheng J, Zhao S, Xu M, Luo Y, Yang Y, Wu Z, Mao L, Wu H, Ling-Hu C, Zhou H, Lin H, Gonzalez-Morales S, Trejo-Saavedra DL, Tian H, Tang X, Zhao M, Huang Z, Zhou A, Yao X, Cui J, Li W, Chen Z, Feng Y, Niu Y, Bi S, Yang X, Li W, Cai H, Luo X, Montes-Hernandez S, Leyva-Gonzalez MA, Xiong Z, He X, Bai L, Tan S, Tang X, Liu D, Liu J, Zhang S, Chen M, Zhang L, Zhang L, Zhang Y, Liao W, Zhang Y, Wang M, Lv X, Wen B, Liu H, Luan H, Zhang Y, Yang S, Wang X, Xu J, Li X, Li S, Wang J, Palloix A, Bosland PW, Li Y, Krogh A, Rivera-Bustamante RF, Herrera-Estrella L, Yin Y, Yu J, Hu K, Zhang Z (2014) Whole-genome sequencing of cultivated and wild peppers provides insights into *Capsicum* domestication and specialization. Proc Natl Acad Sci USA 111:5135–5140

Ramalho do Rego E, Monteiro do Rego M, Cruz CD, Finger FL, Dias Casali VW (2011) Phenotypic diversity, correlation and importance of variables for fruit quality and yield traits in Brazilian peppers (*Capsicum baccatum*). Genet Resour Crop Evol 58:909–918

Rao GU, Ben Chaim A, Borovsky Y, Paran I (2003) Mapping of yield-related QTLs in pepper in an interspecific cross of *Capsicum annuum* and *C. frutescens*. Theor Appl Genet 106:1457–1466

Reddy KR, Kakani VG (2007) Screening Capsicum species of different origins for high temperature tolerance by *in vitro* pollen germination and pollen tube length. Sci Hort 112:130–135

Rehrig WZ, Ashrafi H, Hill T, Prince J, Van Deynze A (2014) CaDMR1 cosegregates with QTL Pc5.1 for resistance to *Phytophthora capsici* in pepper (*Capsicum annuum*). Plant Genome 7:1–12

Richins RD, Micheletto S, O'Connell MA (2010) Gene expression profiles unique to chile (*Capsicum annuum* L.) resistant to Phytophthora root rot. Plant Sci 178 (2):192–201

Rigano MM, Lionetti V, Raiola A, Bellincampi D, Barone A (2018) Pectic enzymes as potential enhancers of ascorbic acid production through the D-galacturonate pathway in Solanaceae. Plant Sci 266:55–63

Rodriguez JM, Berke T, Engle L, Nienhuis J (1999) Variation among and within Capsicum species revealed by RAPD markers. Theor Appl Genet 99:147–156

Ropokis A, Nitatsi G, Kittas C, Katsoulas N, Savvas D (2019) Effect of temperature and grafting on yield, nutrient uptake, and water use efficiency of a hydroponic sweet pepper crop. Agronomy MDPI 9:110

Rosado-Souza L, Scossa F, Chaves IS, Kleessen S, Salvador LFD, Milagre JC, Finger F, Bhering LL, Sulpice R, Aranjo WL, Nikoloski Z, Fernie AR, Nunes-Nesi A (2015) Exploring natural variation of photosynthetic, primary metabolism and growth parameters in a large panel of *Capsicum chinense* accessions. Planta 242:677–691

Rubio JS, Garcia-Sanchez F, Martinez RV (2009) Yield, blossom-end rot incidence, and fruit quality in pepper plants under moderate salinity are affected by K^+ and $Ca2^+$ fertilization. Sci Hort 119:79–87

Sakamoto M, Munemura I, Tomita R, Kobayashi K (2008) Involvement of hydrogen peroxide in leaf abscission signaling, revealed by analysis with an *in vitro* abscission system in *Capsicum* plants. Plant J 56:13–27

Sanchez-Bel P, Egea I, Sanchez-Ballesta MT, Martinez-Madrid C, Fernandez-Garcia N, Romojaro F, Olmos E, Estrella E, Bolarin MC, Flores FB (2012) Understanding the mechanisms of chilling injury in bell pepper fruits using the proteomic approach. J Proteom 75:5463–5478

Sarath Babu B, Pandravada SR, Prasada Rao RDVJ, Anitha K, Chakrabarty SK, Varaprasad KS (2011) Global sources of pepper genetic resources against arthropods, nematodes and pathogens. Crop Protec 30:389–400

Saure MC (2014) Why calcium deficiency is not the cause of blossom-end rot in tomato and pepper fruit—a reappraisal. Sci Hort 174:151–154

Seid A, Fininsa C, Mekete T, Decraemer W, Wesemael WML (2015) Tomato (*Solanum lycopersicum*) and root-knot nematodes (*Meloidogyne* spp.)—a century-old battle. Nematology 17:995–1009

Sezen SM, Yazar A, Eker S (2006) Effect of drip irrigation regimes on yield and quality of field grown bell pepper. Agri Water Manag 81:115–131

Seymour GB, Manning K, Eriksson EM, Popovich AH, King GJ (2002) Genetic identification and genomic organization of factors affecting texture. J Exp Bot 53:2065–2071

Shin R, Han JH, Lee G, Peak KH (2002) The potential use of a viral coat protein gene as a transgene screening marker and multiple virus resistance of pepper plants coexpressing coat proteins of cucumber mosaic virus and tomato mosaic virus. Transgen Res 11:215–219

Shifriss C (1997) Male sterility in pepper (*Capsicum annuum* L.). Euphytica 93:83–88

Singh Y, Sharma M, Sharma A (2009) Genetic variation, association of characters, and their direct and indirect contributions for improvement in chilli peppers. Intl J Veg Sci 15:340–368

Sivakumara TN, Chaudhary S, Kamaraju D, Dutta TK, Papolu PK, Banakar P, Sreevastha R, Singh B, Manjaiah KM, Rao U (2017) Host-induced silencing of two pharyngeal gland genes conferred transcriptional alteration of cell wall-modifying enzymes of *Meloidogyne incognita* vis-à-vis perturbed nematode infectivity in eggplant. Front Plant Sci 8:473

Sood S, Kumar N (2010) Heterotic expression for fruit yield and yield components in intervarietal hybrids of sweet pepper (*Capsicum annuum* L. var. *grossum* Sendt.). SABRAO J Breed Genet 42:106–116

Song EG, Ryu KH (2017) Engineering resistance to a resistance-breaking strain of Cucumber mosaic virus in plants utilizing viral dsRNA. Plant Biotechnol Rep 11:429–438

Stall RE, Jones JB Minsavage GV (2009) Durability of resistance in tomato and pepper to Xanthomonads causing bacterial spot. Annu Rev Phytopathol 47:265–284

Stommel JR, Lightbourn GJ, Winkel BS, Griesbach RJ (2009) Transcription factor families regulate the anthocyanin biosynthetic pathway in *Capsicum annuum*. J Am Soc Hort Sci 134:244–251

SWREC (2019) Southwest Florida Research & Education Center, University of Florida. Vegetable Variety Testing Program. Web document: https://swfrec.ifas.ufl.edu/programs/veg-hort/veg-variety/peppers/. Accessed 31 May 2019

Silvar C, Garcia-Gonzalez CA (2016) Deciphering genetic diversity in the origins of pepper (*Capsicum* spp.) and comparison with worldwide variability. Crop Sci 56:3100–3111

Sun GS, Dai ZL, Bosland PW, Wang Q, Sun CQ, Zhang ZC, Ma ZH (2016) Characterizing and marker-assisting a novel chili pepper (*Capsicum annuum* L.) yellow bud mutant with cytoplasmic male sterility. Genet Mol Res 16: gmr16019459

Sun JT, Cheng GX, Huang LJ, Liu S, Ali M, Khan A, Yu QH, Yang SB, Luo DX, Gong ZH (2019) Modified expression of a heat shock protein gene, CaHSP22.0, results in high sensitivity to heat and salt stress in pepper (*Capsicum annuum* L.). Sci Hort 249:364–373

Thiele R, Muller-Seitz E, Petz M (2008) Chili pepper fruits: presumed precursors of fatty acids characteristic for capsaicinoids. J Agri Food Chem 56:4218–4224

Thies JA, Fery RL (1998) Modified expression of the *N* gene for southern root-knot nematode resistance in pepper at high soil temperatures. J Am Soc Hort Sci 123:1012–1015

Thies JA, Fery RL (2000) Characterization of resistance conferred by the *N* gene to *Meloidogyne arenaria* races 1 and 2, *M. hapla*, and *M. javanica* in two sets of isogenic lines of *Capsicum annuum*. J Am Soc Hort Sci 125:71–75

Thorup TA, Tanyolac B, Livingstone KD, Popovsky S, Paran I, Jahn M (2000) Candidate gene analysis of organ pigmentation loci in the Solanaceae. Proc Natl Acad Sci USA 97:11192–11197

Tijskens LMM, Otma EC, Van Kooten O (1994) Photosystem II quantum yield as a measure of radical scavengers in chilling injury in cucumber fruits and bell peppers. Planta 194:478–486

Tiwari A, Offringa R, Heuvelink E (2012) Auxin-induced fruit set in *Capsicum annuum* L. requires downstream gibberellin biosynthesis. J Plant Growth Regul 31:570–578

Tsaballa A, Pasentsis K, Darzentas N, Tsaftaris AS (2011) Multiple evidence for the role of an Ovate-like gene in determining fruit shape in pepper. BMC Plant Biol 11:46

USDA (2019) Sweet pepper grades and standards. https://www.ams.usda.gov/grades-standards/sweet-peppers-grades-and-standards. Accessed 7 June 2019

Van Eck J (2018) Genome editing and plant transformation of solanaceous food crops. Curr Opinn BioTechnol 49:35–41

Velosco J, Prego C, Varela MM, Carballeira R, Bernal A, Merino F, Diaz J (2014) Properties of capsaicinoids for the control of fungi and oomycetes pathogenic to pepper. Plant Biol 16:177–185

Vicente AR, Pineda C, Lemoine L, Civello PM, Martinez GA, Chaves AR (2005) UV-C treatments reduce decay, retain quality and alleviate chilling injury in pepper. Postharvest Biol Technol 35:69–78

Vidi PA, Kanwischer M, Baginsky S, Austin JR, Csucs G, Dormann P, Kessler F, Brehelin C (2006) Tocopherol cyclase (VTE1) localization and vitamin E accumulation in chloroplast plastoglobule lipoprotein particles. J Biol Chem 281:11225–11234

Visschers IGS, Peters JL, Van de Vondervoort JAH, Hoogveld RHM, Van Dam NM (2019) Thrips resistance screening is coming of age: leaf position and ontogeny are important determinants of leaf-based resistance in pepper. Front Plant Sci 10:510

Wahyuni Y, Ballester A, Sudarmonowati E, Bino RJ, Bovy AG (2011) Metabolite biodiversity in pepper (*Capsicum*) fruits of thirty-two diverse accessions: variation in health-related compounds and implications for breeding. Phytochemistry 72:1358–1370

Wahyuni Y, Ballester A, Tikunov Y, De Vos RC, Pelgrom KTB, Mahrijaya A, Sudarmonowati E, Bino RJ, Bovy AG (2013) Metabolomics and molecular marker analysis to explore pepper (*Capsicum* sp.) biodiversity. Metabolomics 9:130–144

Wahyuni Y, Stahl-Hermes V, Ballester A, De Vos RCH, Voorrips RE, Maharijaya A, Moltthoff J, Zamora MV, Sudarmonowati E, Arisi ACM, Bino RJ, Bovy AG (2014) Genetic mapping of semi-polar metabolites in pepper fruits (*Capsicum* sp.): toward unravelling the molecular regulation of flavonoid quantitative trait loci. Mol Breed 33:503–518

Wall MM, Waddell CA, Bosland PW (2001) Variation in β-carotene and total carotenoid content in fruits of *Capsicum*. HortScience 36:746–749

Wang P, Lu Q, Ai Y, Wang Y, Li T, Wu L, Liu J, Cheng Q, Sun L, Shen H (2019) Candidate gene selection for cytoplasmic male sterility in pepper (*Capsicum annuum* L.) through whole mitochondrial genome sequencing. Int J Mol Sci 20:578

Wang D, Bosland PW (2006) The genes of *Capsicum*. HortScience 41:1169–1187

Wu F, Eannetta NT, Xu Y, Durrett R, Mazourek M, Jahn MM, Tanksley SD (2009) A COSII genetic map of the pepper genome provides a detailed picture of synteny with tomato and new insights into recent chromosome evoluation in the genus Capsicum. Theor Appl Genet 118:1279–1293

Yadav BC, Veluthambi K, Subramaniam K (2006) Host-generated double stranded RNA induces RNAi in plant-parasitic nematodes and protects the host from infection. Mol Biochem Parasitol 148:219–222

Yi GB, Lee JM, Lee S, Choi D, Kim BD (2006) Exploitation of pepper EST-SSRs and an SSR-based linkage map. Theor Appl Genet 114:113–130

Zhu YX, OuYang WJ, Zhang YF, Chen ZL (1996) Transgenic sweet pepper plants from Agrobacterium mediated transformation. Plant Cell Rep 16:71–75

Zhu ZD, Sun BM, Wei JL, Cai W, Huang ZB, Chen CM, Cao BH, Chen GJ, Lei JJ (2019) Construction of a high-density genetic map of an interspecific cross of *Capsicum chinense* and *Capsicum annuum* and QTL analysis of floral traits. Sci Rep 1054

Zhuo K, Chen JS, Lin BR, Wang J, Sun FX, Hu LL, Liao JL (2017) A novel *Meloidogyne enterolobii* effector MeTCTP promotes parasitism by suppressing programmed cell death in host plants. Mol Plant Pathol 18:45–54

Chapter 6
Genome-Assisted Improvement Strategies for Climate-Resilient Carrots

Adam Bolton, Magdalena Klimek-Chodacka, Emily Martin-Millar, Dariusz Grzebelus and Philipp W. Simon

Abstract Carrot is typically categorized as a cool-season vegetable crop that is grown globally with largest per capita production in Europe, but with significant increased production in warmer regions of Asia in the last 50 years. As a high-value vegetable with relatively long postharvest storage life, combined with a high nutritional value attributable to its familiar orange carotenoid pigments, continuing adaptation of carrot to diverse climatic conditions is critical. Traits important to past success and future progress in improving climate resilience depend on the broad genetic diversity of carrot. Classical and modern approaches readily lend themselves to carrot improvement, with significant application of genome-assisted breeding tools expected to expand future prospects of success.

Keywords *Daucus carota* · Cool-season vegetable · Root crop · Climate change · Abiotic stress tolerance · Biofortification

6.1 Introduction

Plants, as sessile organisms, are at mercy of the environment in which they grow and develop. Abiotic stresses, such as heat, drought, and salinity, can result in suboptimal growing conditions for many crops, and although they can survive in environments with abiotic stress , they are likely to experience a reduction in growth and produc-

A. Bolton
Plant Breeding and Plant Genetics Program, Department of Horticulture, University of Wisconsin, Madison, WI, USA

M. Klimek-Chodacka · D. Grzebelus
Department of Plant Biology and Biotechnology, Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow, Krakow, Poland

E. Martin-Millar · P. W. Simon (✉)
Vegetable Crops Research Unit, Department of Horticulture, USDA – Agricultural Research Service, University of Wisconsin, Madison, WI, USA
e-mail: philipp.simon@ars.usda.gov

C. Kole (ed.), *Genomic Designing of Climate-Smart Vegetable Crops*,
https://doi.org/10.1007/978-3-319-97415-6_6

tivity (Bray et al. 2000; Rockström and Falkenmark 2000). It has been suggested that abiotic stressors are the number one cause of crop loss and, on average, reduce yields by 50% or more (Boyer 1982). The amount of abiotic stress affected cropland is expected to increase as many climate models predict a mean global temperature increase of 1–4 °C by 2100 (Pachauri and Reisinger 2007). This increase in temperature will be accompanied by more intense heat waves, drought-like conditions, and an increase in salt accumulation in the soil (Mittler and Blumwald 2010). There is no doubt that abiotic stress is going to be an important issue facing the production of crops worldwide. The development of stress-tolerant cultivars through breeding may be one method to reduce the negative impact of abiotic stress. Until now, relatively little has been written in regards to the relationship between carrot and abiotic stress (Grzebelus 2019).

The origins of carrot were in what is now a warm, dry semiarid region. Best evidence points to Central Asia as the origin of carrot as a root crop only 1100 years ago, with Afghanistan (Mackevic 1929) and then Persia (Laufer 1919) being early sites of carrot cultivation. Molecular evidence also supports a Central Asian origin for carrot (Iorizzo et al. 2013) with a rapid spread and extensive domestication effort to the west of Central Asia into Anatolia, North Africa, and then into Europe by the 1300s (Banga et al. 1957a, b; Banga 1963). Carrot developed somewhat more slowly to the east of Central Asia with its estimated arrival time in China around 1300 (Laufer 1919).

The carrot crop today is grown on 1.2 million hectares and valued at $14 billion globally, placing it in the middle of the top 10 vegetables grown globally (FAO 2019). Global carrot production has increased steadily in the last 50 years, rising at a rate more than compensating for the increase in global population, with the most pronounced increase, greater than eightfold per capita, recorded for Asia (Simon 2019). Consequentially more of the carrot crop is grown in warmer, drier climates now than in the last several hundred years. Carrot breeders have responded to this trend by developing cultivars with improved heat tolerance. The most notable of these is “Brasilia” (Vieira et al. 1983) with not only greater tolerance to heat but also improved Alternaria leaf blight tolerance, making it better suited for climatic conditions of northeastern Brazil and accounting for a significant increase in Brazilian carrot production. Crop improvement to sustain increasing production will require much more attention to global climate trends than it has in the past.

Carrot is a diploid ($2n = 2x = 18$) outcrossing insect-pollinated crop traditionally bred for open-pollinated (OP) cultivar production until cytoplasmic male sterility was discovered in the 1940s and 1950s, when cultivar development for large-scale production shifted to hybrids, which account for the majority of large-scale carrot production today (Simon 2000).

6.2 Prioritizing Climate-Smart (CS) Traits

6.2.1 Flowering Time

Floral initiation in carrot is stimulated by exposure to cool temperature, or vernalization, and is required to trigger the transition from the vegetative crop, which is the commodity grown for commercial production, to flowering and seed production (Linke et al. 2019). Early flowering in the vegetative crop results in fibrous, woody storage roots which are unmarketable, and strong selection against early flowering ("bolting") has been exercised by European and North American breeders since carrots became popular in the 1500s. This strong selection was carried out in geographic regions where winters are too cold for production of a winter crop. In this biennial system, carrots grown as a root crop in one year are stored in root cellars until the next spring, when they are planted for seed production in the second year. Carrot cultivars with this biennial flowering behavior are referred to as "temperate." This is in contrast to carrots grown in warmer climates on an annual cycle starting with production of the vegetative crop during the winter with seed production the following summer after minimal vernalization. This second category of carrots is referred to as "subtropical." The fact that subtropical carrots flower with much less exposure to cold is critical to farmers in warm climates who produce their own seed crop since they have no extended cold season to vernalize carrots, and access to refrigerated cold storage can be limited. Consequentially they must rely on early flowering in the field to be assured of a seed crop. Given their tendency toward early flowering, subtropical carrot cultivars typically flower very readily in temperate root crop production regions and cannot be relied upon for commercial production. Similarly, when temperate carrot cultivars are grown in subtropical carrot crop production regions, access to refrigerated storage is required to be assured of a seed crop and consequentially they may not be suitable if that access is limited.

Given the independent development of temperate and subtropical carrot cultivars in the last 500 years and the role that temperature plays in differentiating them, increasing global temperatures may be expected to require a shift in production regions of temperate cultivars away from the Equator, and a concomitant expanded use of subtropical cultivars, assuming current vernalization requirements remain as they are. As new cultivars are developed, field trialing during development in targeted production regress will be more critical to be assured of reliable performance. The genetic control of vernalization requirement has been elucidated for carrot with a single gene identified by Alessandro and Galmarini (2007) and a second gene described by Wohlfeiler et al. (2019).

6.2.2 Root Characters

Genetic variation in fibrous root growth pattern has not been reported for carrot, but storage root growth, structure, and shape of cultivated carrots have received extensive attention since the storage root is the commodity of commerce. Only recently has genetic control of cultivated carrot root shape been analyzed with several quantitative trait loci (QTLs) controlling diameter, length, and shape (Macko-Podgorni et al. 2017; Turner et al 2017). The relationship between storage root shape and fibrous root growth will be of some interest.

6.2.3 Heat Tolerance

Heat stress can be defined as a rise in temperature above a specific threshold for a period long enough to cause damage to crop growth and development, with that temperature and period of time varying for each species (Wahid et al. 2007). Plant response to heat stress varies depending on the duration of stress, intensity of the temperature, and stage of development. The effects of high temperature can influence many aspects of plant physiology, including reduction of photosynthesis, oxidative stress, reduced plant growth, and inhibition of seed germination (Hasanuzzaman et al. 2013). At extreme temperatures, unrecoverable cellular injury, cell death, and collapse of crucial metabolic processes may occur within a few minutes (Schöffl et al. 1999). Although heat stress can be severely damaging, plants do have the ability to tolerate a certain level of heat stress through physiological and biochemical changes resulting from altered gene expression (Hasanuzzaman et al. 2013).

Of all the physiological aspects of plant growth, photosynthesis is one of the most profoundly affected by heat. It has been suggested that photosystem II (PSII) is the most sensitive element of the photosynthetic machinery (Berry and Bjorkman 1980) and PSII activity may be reduced or halted under heat stress (Morales et al. 2003). High temperatures also negatively affect leaf water status, stomatal conductance, and assimilation of CO_2 (Greer and Weedon 2012). It has been shown that the ability to successfully assimilate CO_2 and continue exchange of gases is directly related to whether the plant is considered heat tolerant. The reduction in CO_2 assimilation under high temperatures is likely attributed to a decrease in Rubisco activity, which is known to begin denaturing at approximately 40 °C (Feller et al. 1998). In crop plants, a reduction in photosynthetic activity reduces the amount of sequestered carbon, decreasing plant growth and adversely affecting yield.

Like Rubisco, many other enzymes important for metabolic functions are also sensitive to high temperatures. As enzymes uncouple, mechanisms normally responsible for scavenging reactive oxygen species (ROS), such as superoxide radical ($\bullet O_{2-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH), begin to degrade, causing an increase in oxidative stress (Asada 2006). These ROS can react with many biomolecules, such as proteins, pigments, lipids, and DNA, and cause a decrease in

cell membrane stability (Rodriguez and Redman 2005; Møller et al. 2007; Huang and Xu 2008). When ROS are allowed to accumulate to a high enough concentration in cells, they may even trigger programmed cell death. Examples of oxidative stress resulting from high temperatures have been demonstrated in many crops, e.g., wheat (Savicka and Shkute 2010), tobacco (Tan et al. 2011), Arabidopsis (Larkindale and Knight 2002), and maize (Gong et al. 1997). Notably, the oxidative stress is not only associated with the heat stress, but rather a general response to many abiotic stresses.

Seed germination is the first stage of plant growth affected by heat stress. The inhibition of seed germination, either complete prevention or rate reduction, typically occurs via the induction of abscisic acid (ABA), which is a known stress response hormone (Shinozaki and Yamaguchi-Shinozaki 2006). It has been suggested that as ABA increases in the seed as a response to stress, it limits the availability of energy and nutrients, thus preventing the seed from having the energy required to germinate (Garciarrubio et al. 1997). At extreme temperatures, germination may be completely inhibited due to cell death and unrecoverable embryo damage, as has been demonstrated in wheat seeds (Essemine et al. 2010).

Heat tolerance, sometimes called thermotolerance, is defined as the ability of a plant to grow under high temperatures and produce economically viable yields and is a highly complex trait that varies greatly both among and within species (Hasanuzzaman et al. 2013). Plants demonstrate different mechanisms for dealing with high temperatures depending on the duration and intensity of the heat stress. Some important mechanisms of heat tolerance include the production of antioxidants to combat oxidative stress (Maestri et al. 2002), the accumulation of compatible osmolytes to increase intracellular osmolarity (Sakamoto and Murata 2002), an increase in the chlorophyll *a:b* ratio and carotenoid content to maintain PSII function (Camejo et al. 2006), and the production of heat shock proteins (HSPs) (Bowen et al. 2002). There are multiple mechanisms by which HSPs aid in combating heat stress. HSPs help proteins normally disrupted by high temperatures maintain their shape and function, shuttle proteins aid in protein translation and translocation, reactivate denatured proteins, and protect photosystems from oxidative damage (Neta-Sharir et al. 2005; Stetler et al. 2010).

Carrot, as a cool-season crop, may be particularly sensitive to high temperatures, which is one of the major abiotic factors limiting all stages of growth (Landjeva et al. 2008). Although relatively little work has been undertaken regarding carrot thermotolerance, a few mechanisms and candidate genes have been suggested. The first, alternative oxidase (AOX), is an enzyme that is noted to relieve oxidative stress caused by the formation of ROS (Amirsadeghi et al. 2007). The carrot genome carries three *AOX* genes, one representing *AOX1* and two *AOX2* paralogs (Campos et al. 2009). The *AOX* genes might be responsible for relieving environmentally induced oxidative stress by limiting the formation of ROS in the mitochondria (Nogales et al. 2016). Indeed, the expression of carrot *AOX* was markedly affected by temperature changes, e.g., *DcAOX1* was highly upregulated when ambient temperature raised from 21 °C to 28 °C (Campos et al. 2016). Possibly, allelic variability within *DcAOX1* could have an impact on the heat stress tolerance and the gene could be a target for marker-assisted selection (Nogales et al. 2016).

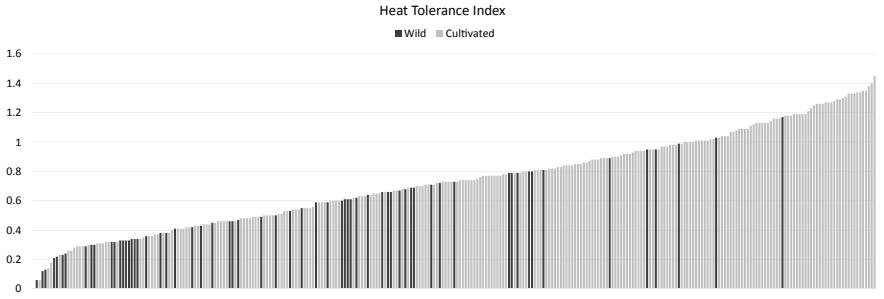

Fig. 6.1 Variation in the heat tolerance index for a collection of wild and cultivated carrot germplasm accessions. Each bar represents a different germplasm accession reported in Bolton et al. (2019)

DcHsp17.7, a carrot HSP, was reported by Malik et al. (1999) as being capable of increasing plant tolerance to high temperatures, up to 42 °C. Park et al. (2013) showed that it was rapidly synthesized in response to the heat treatment, remained abundant two days later, and subsequently decayed. Night exposure to heat showed a more pronounced effect on the accumulation of DcHsp17.7. Several other HSPs were shown to be upregulated by heat stress (Huang et al. 2015).

With the development of carrot cultivars targeted for production in a warmer climate, the influence of elevated temperature on early crop growth has been evaluated. Nascimento et al. (2008) and Bolton et al. (2019) observed seed germination to be reduced with elevated temperatures where, relative to the control temperature of 24 °C, germination of most carrots was reduced at least 50% up to 35 °C, but several OPs evaluated exhibited no significant reduction in germination at 35 °C compared to 24 °C (Fig. 6.1). At 37.5 °C, only "Brasilia" seed germinated among the cultivars tested, but at a rate less than 10%. Temperature levels under which the carrot root crop can survive during stand establishment and crop growth beyond germination have not been reported.

Beyond production of the root crop, heat tolerance may also play an important role in carrot seed production. Broussard et al. (2017) exposed flowering carrots to "cool", "average," and "warm" greenhouse conditions and observed reduction in volatile terpenoid production and nectar quality, which was conjectured to reduce attractiveness of insect pollination. Since adequate seed production is critical to sustain crop production, expanded studies on the effects of climatic effects on the reproductive phase of the carrot life cycle will be of great importance.

6.2.4 Cold Tolerance

Climate change can include temperature fluctuations not only above recent averages, but also temperatures below recent averages. Carrot is generally regarded as cold-hardy and able to recover from cold temperatures as low as −8 °C. Beyond leaf

damage, cold temperatures cause taproot cracking in carrot. Palta and Simon (2004) observed variation among breeding stocks for leaf and root damage, and exercised selection for reduced incidence of taproot cracking. Two frost tolerant hybrid cultivars were developed and released.

6.2.5 Salinity Tolerance

There are two distinct mechanisms by which high levels of salinity impede plant growth and development. The first occurs when high levels of salt in the soil create an osmotic effect that reduces the ability of the seeds and roots to pull water from the surrounding environment and into the plant tissue, creating drought-like symptoms such as reduced cell expansion in the leaves, roots, and seeds (Munns and Tester 2008). The second mechanism is the accumulation of salts to toxic levels within the plant tissue, interfering with major biological processes critical to plant growth, and creating ionic stress that often results in tissue death. For example, the accumulation of Na^+ can reduce the functionality of chlorophylls, carotenoids, and essential photosynthetic enzymes (Davenport et al. 2005), which can result in oxidative stress caused by the formation of ROS (Apel and Hirt 2004). Mineral nutrient deficiencies can occur when Na^+ competes for transport protein sites that normally uptake critical macronutrients such as K, N, and P (Carillo et al. 2011). It was first suggested by Munns et al. (1995) that both of these effects on plant growth and survival occur in a two-phase model. In Phase 1, high levels of salts create osmotic stress that tends to decrease growth rate, followed by the toxic ionic effects of Phase 2, which are often more harmful (Carillo et al. 2011). During the second phase, ions are transported through the xylem and deposited in the leaf blade where they accumulate and can kill older leaves. These two phases of salinity stress have a greater negative effect on the shoots, which tend to be less tolerant than the roots (Munns and Tester 2008). Response to salinity varies with developmental stage, or ontogeny; the most sensitive and critical stages of the plant life cycle are typically germination, seedling establishment, and flowering (Flowers 2004). Tolerance is also dependent on other environmental conditions such as soil temperature, soil moisture, physical properties of the soil, air temperature, and humidity (Munns and James 2003). Salt-tolerant plants (halophytes) have developed mechanisms to overcome the accumulation of these toxic ions through multiple salinity tolerance mechanisms that each have been found to be under independent genetic control.

Salinity tolerance mechanisms can be broken up into three main categories: (1) tolerance to osmotic stress, (2) Na^+ exclusion from the leaves, and (3) tolerance of tissue to Na^+ accumulation (Munns and Tester 2008). Osmotic stress tolerance is typically conferred by increased water-use efficiency and/or osmotic adjustment via increased proline or soluble sugar accumulation (Munns 2005). Na^+ exclusion from the leaves starts with the selective exclusion of Na^+ over K^+ by the roots (Munns and Rawson 1999) or by efflux of Na^+ back out into the soil rather than transport into the xylem (Tester and Davenport 2003). In many species, salt exclusion is strongly

correlated with salt tolerance and has been shown to have a wide range of natural variation among species (Yeo and Flowers 1986; Munns and James 2003; Tester and Davenport 2003). Tissue tolerance of Na^+ accumulation occurs when plants are able to compartmentalize Na^+ into the vacuole to prevent reaching toxic levels in the cytoplasm. This also requires the synthesis of solutes in the cytoplasm to maintain osmotic balance with the vacuole (Tester and Davenport 2003). These solutes (e.g., proline, sucrose, glycine betaine, and mannitol) are compounds that do not interfere with normal biochemical functions (Shomer-Ilan et al. 1991). Several candidate genes related to these salinity tolerance mechanisms have been identified and could be combined to give higher levels of salinity tolerance in many crops (Yeo and Flowers 1986). Major genes have been identified that contribute to salinity tolerance, but the functions in which they are involved (ion transport, protein synthesis, hormone signaling) are complex, and consequently it is not surprising that much of adaptation to salinity stress, as well as to other abiotic stresses, is governed by quantitative variation (Sreenivasulu et al. 2007). Phenotypic parameters for screening salinity tolerance vary depending on the salinity concentration, duration of stress, and the developmental stage of the plant (Shannon 1985). The strictest measure of tolerance is whether a genotype has the ability to survive through the completion of its life cycle at high salinity levels. A genotype that can survive from seed germination, through seedling establishment, and on to flowering is considered tolerant in the most absolute sense. This level of tolerance may not be necessary for most crop species, but even relatively low levels of salinity can reduce biomass accumulation and yield significantly. For many crop species, biomass and yield reduction under salinity stress are useful criteria for quantifying tolerance but do not provide insight into the mechanisms conferring tolerance (Bado et al. 2016).

As mentioned previously, leaves are often more sensitive to salinity stress than roots, and thus have been a focus of phenotyping procedures. Leaf damage can be easily observed as necrosis or yellowing and has been successfully used to phenotype salinity stress response in wheat and barley (Richards et al. 1987), and rice (Gregorio et al. 1997). Scoring of leaf wilting, another leaf trait, has been shown to be effective in adzuki bean, *Vigna angularis* L. (Yoshida et al. 2016), but can be inaccurate due to the subjectivity of scoring.

Phenotyping at seed germination is a relatively easy and fast (7–21 days) measurement that is critical for plants in saline conditions and found to be controlled by other genes than those controlling leaf damage. The most frequently used measurement for germination tolerance is relative percent germination in salinity: percent germination under a defined salinity concentration divided by percent germination without salinity. Relative percent germination as a tolerance trait has been evaluated in many species including *Triticum durum* L. (Almansouri et al. 2001), *Arabidopsis thaliana* L. (DeRose-Wilson and Gaut 2011), *Zea mays* L. (Radić et al. 2007), and *Pisum sativum* L. (Shahid et al. 2012). Independent screening for specific traits related to all three physiological mechanisms of salt tolerance (Na^+ exclusion, K^+/Na^+ discrimination, and tissue tolerance) has been argued as the best method for maximizing the genetic improvement of salt tolerance (Noble and Rogers 1992; Munns and Rawson 1999; Munns and James 2003; Yoshida et al. 2016). Each of these traits is frequently

controlled by specific genes and therefore there is potential to pyramid these traits together to increase tolerance above what may be normally found in one genotype (Noble and Rogers 1992). Harvesting root and shoot tissue grown with and without salinity stress and analyzing it for Na^+ and K^+ concentrations allow for identification of mechanisms, whether it be salinity exclusion or tolerance, that the plant is utilizing to cope under the stress. Comparing these concentrations with percent biomass reduction under stress allows for the identification of tolerant genotypes/accessions and the mechanisms of tolerance utilized (Munns and James 2003). Quantifying the concentration of the ions can be done by studying the "Ionome" of plants (Baxter 2009).

Carrot, as a salt-sensitive glycophytic plant, has long been observed to be one of the most salt-sensitive vegetable crops (Bernstein and Ayers 1953; Maas and Hoffman 1977). Carrot yield, measured in terms of root biomass, declines approximately 14% for every unit increase in salinity past 1.0 dS m^{-1} threshold, which is much lower than the defined threshold, 4 dS m^{-1}, for a saline soil. Carrot seed germination and seedling establishment (Fig. 6.2) also suffer greatly from increased salt concentrations in the soil (Schmidhalter and Oertli 1991). Both the capacity for total seed germination and rate of germination are decreased greatly under salinity stress with these effects becoming greater as concentration of salt increases (Kahouli et al. 2014). Salinity stress has also been noted to cause reduced rates of photosynthesis and stomatal conductance in carrot (Gibberd et al. 2002).

Similar to heat stress tolerance, relatively little work has been undertaken to identify mechanisms of salt tolerance in carrot, but some have been suggested. Changes in the enzymatic and nonenzymatic antioxidant defense system of carrots under salt stress have been demonstrated by Bano et al. (2014) suggesting that increase in glycine betaine, ascorbate, and other antioxidants may place a role in salt stress tolerance. Possibly, phytoene synthase 2 (DcPSY2) may also be involved in the reaction of carrots to salinity. DcPSY2 is one of the key proteins in the carotenoid biosynthesis pathway in carrot roots (Fuentes et al. 2012; Wang et al. 2014). In turn, carotenoids are precursors of ABA. Simpson et al. (2018) showed that the salinity stress and

Fig. 6.2 Carrot plants at 42 days of growth without (left) and with (right) 150 mM NaCl added to irrigation solution

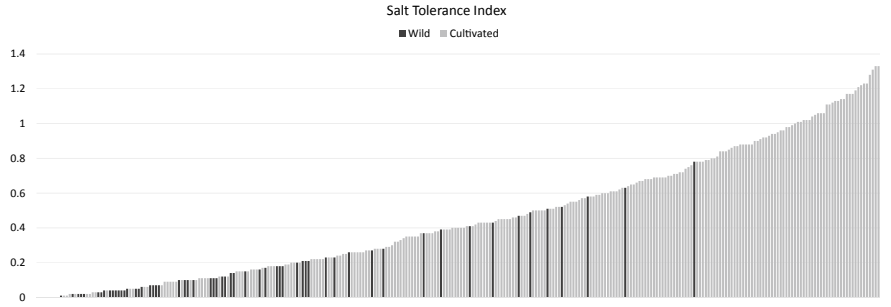

Fig. 6.3 Variation in the salt tolerance index for a collection of wild and cultivated carrot germplasm accessions. Each bar represents a different germplasm accession reported in Bolton and Simon (2019)

ABA upregulate *DcPSY2* through binding of DcAREB3 transcription factor to ABA responsive elements (A located in the promoter of *DcPSY2*).

Since carrot is irrigated in much of its global production, and rising levels of salinity is an increasing problem, an assessment of genetic diversity in carrot germplasm for salinity tolerance can provide important insights into future prospects for greater salinity tolerance in the carrot crop. Kahouli et al. (2014) evaluated 10 carrot cultivars and observed variation indicating a genetic component to carrot salinity tolerance. Bolton and Simon (2019) evaluated 294 diverse cultivated and wild carrot accessions and confirmed broad variation for tolerance to 150 mM NaCl during germination (Fig. 6.3), including breeding stocks and OPs. The observation of relatively high levels of salinity tolerance in cultivated germplasm provides an optimistic outlook for future CS carrot crop improvement.

6.2.6 Drought Stress

Reduced rainfall and changes in rainfall patterns are very dangerous for agriculture (Fahad et al. 2017). Typical symptoms of drought stress in plants are reduced leaf water potential and decreased cell growth, which adversely affect both the plant growth as well as a range of physiological or biochemical processes, including photosynthesis, nutrient metabolism, respiration, and chlorophyll synthesis (Hussain et al. 2018). Thaumatin-like proteins are included in a group of pathogenesis-related proteins. However, these proteins are also involved in response to abiotic stresses. In carrot, a *dcTLP* gene encoding a thaumatin-like protein (TLP) was reported to be upregulated upon dehydration, independently from the developmental stage and not regulated by ABA, salicylic acid or jasmonic acid. Possibly it is one of the elements conferring physiological adaptation of carrots to drought, in combination with other drought-induced genes (Jung et al. 2005). A small HSP, DcHsp17.7, referred to in the

heat stress section, was also shown to accumulate in carrots suffering from osmotic stress (Ahn and Song 2012).

Few reports of carrot growth under drought stress have been published. Sorensen et al. (1997) reported yield reduction and significant changes in sugar content and other components of nutrient composition in carrot due to drought, and they noted variation among cultivars tested to suggest a genetic component to drought tolerance in carrots. Given the recurring shortage of rainfall and dwindling access to adequate quality irrigation water in recent decades, detailed field performance information evaluating the effects of drought on carrot productivity will be valuable.

6.2.7 Disease and Pest Resistance

Several diseases challenge carrot growers (du Toit et al. 2019; LeClerc et al. 2019). The most widespread foliar disease globally is Alternaria leaf blight which especially threatens carrot production in humid climates. Genetic analyses have identified several QTLs contributing to the resistance response (LeClerc et al. 2015, 2019) and many breeding programs are selecting for improved resistance. Root-knot nematodes are another significant pest of carrot, and resistance genes to protect against *Meloidogyne incognita* and *M. javanica* have been identified (Simon et al. 2000; Parsons et al. 2015). Both Alternaria leaf blight and root-knot nematodes are widespread challenges in subtropical carrot production regions, so durable resistance is critical as climate-resilient carrots are developed. The most important postharvest disease in carrot is cavity spot, caused by several *Pythium* species. Variation in *Pythium* resistance is observed among cultivars and breeding stocks. The relatively long potential postharvest season storage is an attractive feature of carrots, but to fully realize that potential, resistant cultivars will be important. Numerous other diseases of carrots have been identified, and as production expands in subtropical carrot-growing regions, several diseases may become more important (du Toit et al. 2019).

6.2.8 Insect Resistance

Carrot fly (*Psila rosae*) is the most important insect pest that damages the carrot crop (Collier and Finch 2009). Partial resistance has been identified but additional sources of resistance are expected to be necessary. Carrot fly is primarily a problem in cooler growing regions of Northern Europe and Canada. If temperate carrot production moves north in these regions with the advance of warmer climates carrot fly could become more of a problem.

Insects vector several microbial diseases of carrot (Groves et al. 2019). Carrot psyllid-vectored diseases may pose especially challenging threats (Nissinen et al. 2012) and warmer climates have been projected to potentially heighten their likely impact.

6.2.9 *Antioxidants and CS Carrots—A Role in Plant Stress Tolerance and Human Health*

6.2.9.1 Antioxidant Response to Environmental Stress

While much research has been focused on the antioxidant content of carrots from a human nutritional perspective, little has been done on the antioxidant activity of carrots prior to harvest. The most prominent antioxidants in carrot are the pigments that determine the many possible colors of their roots; the carotenoids, which include alpha- and beta-carotene, lycopene, and lutein, and confer orange, red, and yellow pigmentation, respectively, and the anthocyanins, which confer purple pigmentation. These photosynthetic pigments, which only occur in the shoot in most plants, accumulate in carrot roots due to a defect in light sensing that allows the carotenoid, and possibly also the anthocyanin, metabolic pathways to be expressed in darkness (Iorizzo et al. 2016). Both carotenoids and anthocyanins function as nonenzymatic low molecular metabolites in enzymatic antioxidant systems (Gill and Tuteja 2010).

Many known antioxidants are synthesized in the carotenoid biosynthetic and its related pathways. Just upstream of carotenoid biosynthesis is the synthesis of terpenoids, which function as antioxidants (Graßmann 2005) and contribute to the distinctive flavor of carrots (Keilwagen et al. 2017). Among these, isoprene, a hemiterpene found in carrot roots (Duke 1992), is known to increase thermotolerance in kudzu (Singsaas et al. 1997), while monoterpenes, too, improve thermotolerance and protect plants against oxidative stress (Gill and Tuteja 2010). The first committed step of the carotenoid biosynthetic pathway is catalyzed by phytoene synthase, and the overexpression of its two isoforms in carrot is responsible for orange root pigmentation (Wang et al. 2014). Expression of the second isoform in carrot, *DcPSY2*, is induced by salt stress and the phytohormone ABA, which is synthesized downstream of carotenoids and plays a major role in mediating abiotic stress tolerance across plant species (Simpson et al. 2018), indicating a direct link between orange root pigmentation and abiotic stress response. ABA has also been shown to specifically enhance antioxidant response in several other diverse plant species, including intertidal seaweed species (Guajardo et al. 2016), Malabar plum (*Syzygium cumini*) (Choudhary et al. 2012), and pumpkin-grafted cucumber seedlings (Shu et al. 2016). The carotenoids synthesized in this pathway are known for protecting plants against photooxidative stress by efficiently scavenging singlet oxygen and peroxyl radicals (Stahl and Sies 2003), and among them, lycopene is the strongest antioxidant in terms of singlet oxygen quenching (Di Mascio et al. 1989). While growing carrot roots are mostly shielded from the sun, these pigments can protect plants from oxidative stress in general (Sies and Stahl 1995), which can be induced by drought, heat, and salinity stresses (Krishnamurthy and Rathinasabapathi 2013).

Anthocyanins, ubiquitous and abundant in purple carrots, are synthesized in the flavonoid pathway in response to abiotic stress as well as other stimuli. The production of anthocyanins often correlates with increased stress tolerance, and the proposed mechanisms for this include quenching of ROS, photoprotection, and stress

signaling (Kovinich et al. 2015). The accumulation of anthocyanins may also inhibit foliar senescence under nutrient deficiency (Landi et al. 2015), a condition which can be induced by salt stress (Acosta-Motos et al. 2017). In fact, salt stress has been shown to stimulate anthocyanin accumulation in higher plants (Eryılmaz 2006). In a small comparative study of black and orange carrots from Cuevas Bajas, Spain, the black carrots displayed a higher antioxidant activity than the orange, potentially due to higher total phenolic content, including anthocyanins (Algarra et al. 2014). This could also be due to higher antioxidant capacity of anthocyanins over carotenoids, or to higher total pigment content, or perhaps even to synergistic antioxidant effects of anthocyanins and carotenoids.

While there is an evident correlation between photosynthetic pigment accumulation and abiotic stress response, a causative relationship between pigment content and tolerance has not yet been determined. However, while most crops are more stress-sensitive than their wild progenitors, Bolton and Simon (2019)demonstrated that wild *D. carota*, which is predominantly white-rooted, is significantly less salt-tolerant, at least at the germination stage, than variously colored Turkish and Indian landraces of carrot, suggesting that these root pigments may directly enhance abiotic stress tolerance of carrot. This would accord with the biological principle of xenohormesis, which dictates that plants subjected to environmental stresses produce bioactive compounds that provide stress resistance to consumers (Hooper et al. 2010); carrots that thrive under abiotic stress conditions would then contain more of the antioxidant pigments that benefit human consumers, which has been the primary focus of carrot antioxidant studies.

There have been few reports of environmental effects on the accumulation of carrot carotenoids, anthocyanins, or other antioxidant compounds. Barnes (1936) reported that both root size and carotene content were higher at 17–19 °C than at either 11–13 °C or at 23–25 °C soil temperatures, and anecdotal information on carrot color intensity from season to season supports the conclusions from these early studies. Given their involvement in plant growth and response to stress and their importance in human nutrition, more information on antioxidant accumulation will be valuable.

6.2.9.2 Antioxidants and Human Health

The pigments familiar to consumers in orange carrots are provitamin A carotenoids, while lutein in yellow carrots, lycopene in red carrots, and anthocyanins in purple carrots also have important roles in human nutrition as antioxidants promoting eye health and protecting against certain forms of cancer (Simon et al. 2008; Arscott and Tanumihardjo 2010). Wide variation for pigment content and composition can be found among diverse cultivated carrots and a wide range of research has been published on the genetic control of carrot pigments (Table 6.1; reviewed by Cavagnaro and Iorizzo 2019; Simon et al 2019). Given the rise in carrot production in regions of the world with significant micronutrient deficiency and stressful climates for crop production, additional research on the antioxidants of carrot in global agricultural settings will have multiple significant implications.

Table 6.1 Mapped simply inherited traits and QTLs of carrot

Gene symbol	Trait	References
Growth and reproductive biology		
Vrn1	Vernalization	Alessandro et al. (2013)
Rf1	Nuclear restorers of CMS	Alessandro et al. (2013)
Gum1-2, Mar1-2, Gad1-2	Novel cytoplasms and sterility	Borner et al. (1995)
STS1-STS6	Petaloid male sterile and fertile cytoplasm	Nakajima et al. (1999)
14 primer pairs		Bach et al. (2002)
Phenl	Small, dark green, annual	Schulz et al. (1994)
COLA	Compressed lamina	Budahn et al. (2014)
YEL	Yellow leaf	Budahn et al. (2014)
cult	Root thickening	Macko-Podgorni et al. (2017)
5, 4, and 3 QTLs 1, 5, and 3 QTLs 6, 2, and 2 QTLs	Shoot height, biomass, area Petiole number, width, and length Root length, biomass, and area	Turner et al. (2018)
Disease and pest resistance		
3 QTLs	Alternaria leaf blight	LeClerc et al. (2019)
11 QTLs		LeClerc et al. (2015)
Mj-1	*M. javanica* root-knot nematodes	Boiteux et al. (2000, 2004)
Mj-2	*M. javanica* root-knot nematodes	Ali et al. (2013)
7 QTLs	*M. incognita* root-knot nematodes	Parsons et al. (2015)
Nutritional quality and flavor		
Y	Yellow xylem and phloem	Just et al. (2009), Iorizzo et al. (2016)
y_2	Differential orange phloem/xylem	Bradeen and Simon (1998), Just et al. (2009), Yildiz et al. (2013), Ellison et al. (2017)
16 QTLs	Carotene content	Santos and Simon (2002)
Or	Carotene content	Ellison et al. (2018)
P_1	Root anthocyanins	Vivek and Simon (1999), Yildiz et al. (2013), Cavagnaro et al. (2014)
P_3	Root and petiole anthocyanins	Cavagnaro et al. (2014)
Raa1	Acylated anthocyanins	
15 QTLs	Anthocyanin content	
30 QTLs	Volatile terpenoid content and composition	Keilwagen et al. (2017)
Rs	Reducing sugar	Vivek and Simon (1999, Yau et al. (2003, 2005)

6.3 Genetic Resources

The primary gene pool of carrots includes cultivated carrot (*Daucus carota* ssp. *sativus*) and wild carrot (*Daucus carota* ssp. *carota*). Their range of genetic and phenotypic diversity is broad, and they are freely intercrossable (Peterson and Simon 1986; Simon 2000). A secondary gene pool for carrot includes those North African and eastern Mediterranean species with the same chromosome number as carrot, 2n = 2x = 18. Interspecific crosses with species in the secondary pool have not been reported. The genus *Daucus* includes approximately 40 species (Banasiak et al. 2016; Spooner 2019) and may be considered a tertiary gene pool of carrots. A relatively extensive collection of *Daucus* germplasm has been collected (Allender 2019), but wild carrot germplasm is not well represented (Castaneda-Alvarez et al. 2016).

6.4 Classical Genetics and Breeding

6.4.1 Genetics

Carrot is not a model organism for genetic studies and genetic analysis of carrot has not been extensively pursued. Seed production requires time and experience beyond production of the root crop to vernalize plants and produce the seed crop. Furthermore, carrot flowers are very small and each flower produces a maximum of two seeds, making pollinating by hand challenging and not very rewarding. In contrast, insect pollination of carrot umbels with houseflies or blue bottle flies can yield several hundred seeds per plant.

Twenty single genes controlling phenotypic traits were reported for carrot by 1985 and no linkages had been identified. The carrot chromosome number was known but no genes were associated with chromosomes. Isozyme analysis had been used for taxonomic research but not genetic analysis (Peterson and Simon 1986). The advent of the use of biochemical and molecular markers in the 1990s stimulated more extensive carrot genetic analysis.

6.4.2 Breeding

Shorter term carrot breeding objectives focus on improving disease and pest resistance, storage root appearance, color, flavor, and population uniformity (Peterson and Simon 1986; Simon and Goldman 2007; Simon et al. 2008; Simon and Grzebelus 2019). The popularity of hybrid cultivars stems from the uniformity that they can afford, and their proprietary nature stimulated an expanded interest in initiating carrot breeding programs among seed companies (Simon 2000). Longer term carrot breeding objectives have included abiotic stress tolerance and introgression of traits

between temperate and subtropical breeding pools. Introgression of traits from wild carrot into cultivated breeding stocks can be expected to be a much longer term effort.

6.5 Diversity

6.5.1 Phenotypic Diversity

Cultivated carrot varies widely in phenotypic diversity (Fig. 6.4) as reflected in traits ranging from storage root color, shape, and flavor to leaf morphology, size, and pubescence and to umbel shape, petal color, and pollinator attractiveness. Wild carrot also varies widely in most of these traits except that roots are typically narrower, more fibrous with prominent lateral roots, and root color is white or very pale yellow. Diversity analysis of carrot has typically included an evaluation of not only phenotypic diversity but also genotypic diversity as molecular genetic markers were developed.

Fig. 6.4 Variation in carrot color attributable to carotenoid (orange, red, yellow) and anthocyanin (purple) pigments (Photo by Steve Ausmus, USDA/ARS)

6.5.2 Genotypic Diversity

Several studies have utilized diverse collections of wild and cultivated carrots to evaluate genetic diversity, geographic substructure, and patterns of domestication in carrot. Bradeen et al. (2002) utilized less than 200 molecular markers, primarily AFLPs and ISSRs, and observed clear separation between wild and cultivated carrots, but no structure among cultivated carrots evaluated based on storage root color or shape, or geographic origin. However, based on an evaluation utilizing 4,000 SNPs, Iorizzo et al. (2013) distinguished not only wild carrots from cultivated, but also found that wild carrots from Central Asia (Afghanistan, Uzbekistan) were genetically most similar to cultivated carrots, than were wild carrots from other geographic origins. This study also confirmed that cultivated carrots from east of this Central Asian center of domestication grouped separately from cultivated carrots west of Central Asia. Utilizing additional markers and diverse carrots, Ellison et al. (2018) confirmed these observations and also noted an additional cluster among cultivated carrots that included western hybrid carrots of the Imperator type. The differentiation between eastern and western geographic origins of cultivated carrots in these studies agrees with historical records indicating a separate historical development of carrot as a root crop progressing west from Central Asia around 900 through Anatolia and North Africa to southern Europe by the 1100s, while the first records of carrot in China were in the 1300s and Japan in the 1700s (Banga et al. 1957a, b; Banga 1963).

A small reduction in overall genetic diversity, if any, has been observed during the domestication of carrot. $H_e$in both wild and cultivated carrots was 0.32 in the Iorizzo et al. (2013) study, while genetic diversity was 3.25×10^{-5} and 3.13×10^{-5} for these respective groups in the Ellison et al. (2018) study. This may reflect the likely recurring introgression of wild carrot, thought to be widespread throughout temperate regions of Europe and Asia thousands of years ago, into cultivated carrots during domestication.

6.6 Association Mapping

Few genome-wide association studies (GWAS) have been reported for carrot (Iorizzo et al. 2019a). An evaluation of 109 SNPs distributed in 17 carotenoid biosynthesis genes in a collection of carrots varying in carotenoid-based root color by Jourdan et al. (2015) found orange color and carotenoid content to be associated with two of these genes, *ZEP* and *CRTISO*. With the availability of the carrot genome sequence (Iorizzo et al. 2016), Keilwagen et al. (2017) associated 15 volatile flavor compounds found in carrot roots with 30 QTLs. Ellison et al. (2018) detected genomic regions that differentiated wild and cultivated carrots. Three genes previously known to be associated with carotenoid accumulation and composition in orange carrots—*Y, Y_2*, and carotene hydroxylase—were included in the genomic regions mapped, as was a

candidate gene for root thickening (Macko-Podgorni et al. 2017), and a previously unidentified gene associated with carotenoid accumulation, *Or*.

The ability to detect genomic regions in GWAS depends on the occurrence of linkage disequilibrium (LD), with rapid decay expected in an outcrossing crop like carrots. In fact, Ellison et al. (2018) observed rapid decay rates, <1 kb, in wild carrots and moderate decay, <10 kb, in cultivated carrots. As in other crops, LD values vary across the genome and even slower decay is observed around genomic regions under selection during domestication in carrot. With the rapid LD decay observed in carrot, high levels of SNP coverage will benefit GWAS in carrot.

6.7 Molecular Mapping

Genetic linkage in carrot was first reported by Westphal and Wricke (1991) with four linkage groups identified mapping 12 isozyme markers. By the middle of the 2000 to 2010 decade, four additional reports mapped approximately 900 more markers, primarily RFLP, RAPD, and AFLPs, and four morphological traits (reviewed by Bradeen and Simon 2007).

Progress in molecular mapping has accelerated since 2000. QTL analysis was first reported for carrot in 2002, first extensive SSR map and FISH map in 2011, SNP map in 2013, and both DArT map and GBS map in 2014 (reviewed by Iorizzo et al. 2019a).

Early carrot genetic maps were usually derived from F_2 populations developed from unrelated parents. Mapped traits that contribute to climate resilience include floral initiation; male sterility, important for hybrid production; leaf growth, important for weed competitiveness; storage root morphology, size, and shape; leaf blight and nematode resistance; nutritional pigments; and sugars that contribute to culinary quality (6.1). As an example, the QTL map for *Meloidogyne incognita* resistance (Fig. 6.5) and table describing the contributions of those QTLs to resistance (Table 6.2) are included.

6.8 Marker-Assisted Breeding

Molecular markers have been developed for carrot root color and sugar type, and root-knot nematode resistance. Bradeen and Simon (1998) identified linkage between the Y_2 locus, which conditions carotene accumulation in the carrot xylem core, and six linked AFLP markers. A simple codominant PCR-based marker ~2 cM from Y_2 was developed. Ellison et al. (2017) refined markers for Y_2 by developing cleavage amplified polymorphic sequences <<1 cM away that were very accurate in predicting orange and non-orange phenotypes. Yau developed markers within the candidate gene for the *Rs* locus that controls the type of sugar stored in the storage root (Yau and Simon 2003) and effectively selected for sugar type of mature plants based on

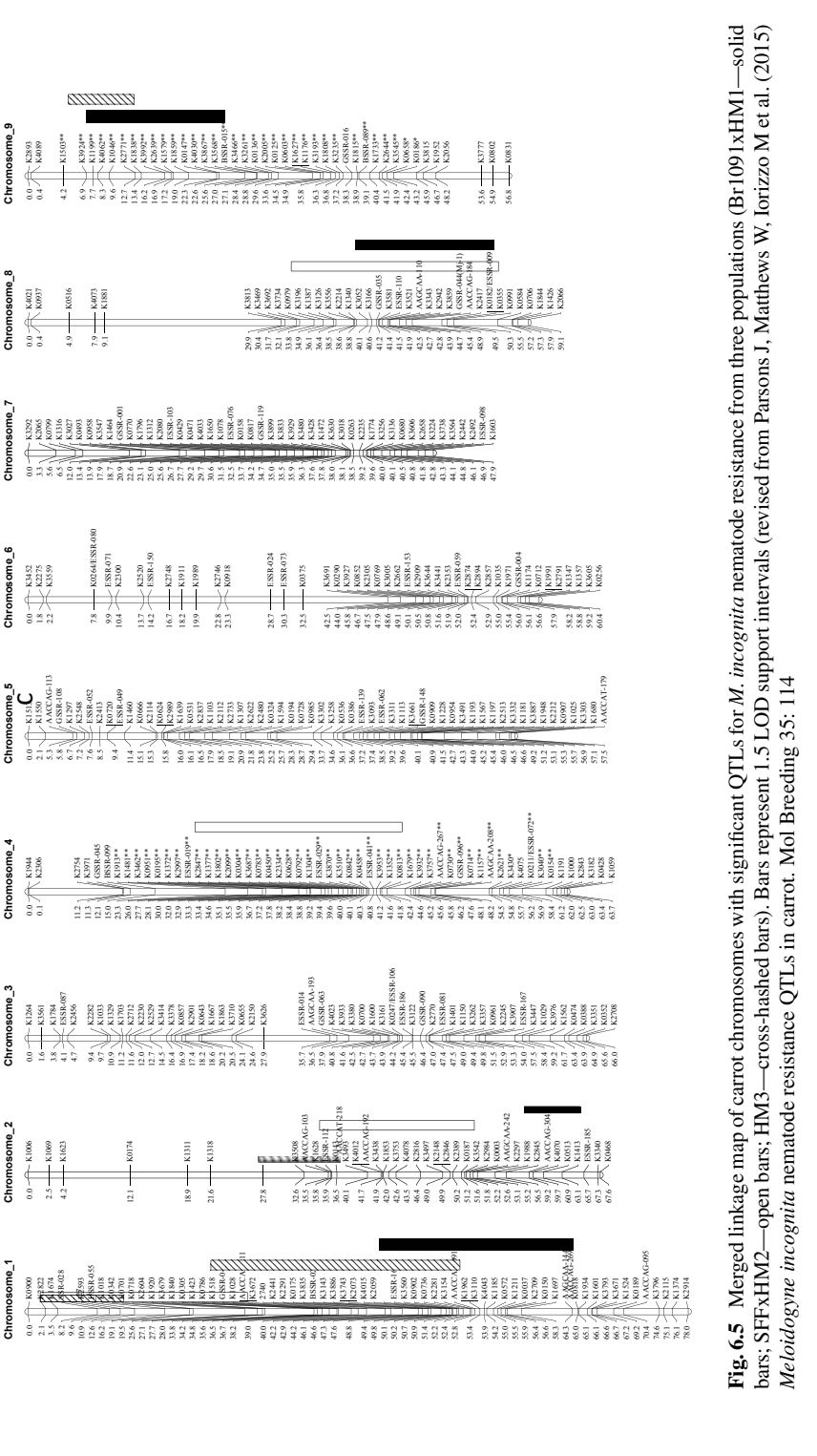

Fig. 6.5 Merged linkage map of carrot chromosomes with significant QTLs for *M. incognita* nematode resistance from three populations (Br1091xHM1—solid bars; SFFxHM2—open bars; HM3—cross-hashed bars). Bars represent 1.5 LOD support intervals (revised from Parsons J, Matthews W, Iorizzo M et al. (2015) *Meloidogyne incognita* nematode resistance QTLs in carrot. Mol Breeding 35: 114

Table 6.2 Chromosomal locations of QTL conferring *M. incognita* nematode resistance in the three carrot mapping populations and their contribution to resistance

(Mapping population) chromosome	QTL	Position (cM)	LOD	% VE[a]	Resistant parent	1.5 LOD[b]	Additive effect[c]
(Br1091× HM)							
1	*Mi-BrHM1-C1-Q3*	67.2	3.9	6.1	B1091	52–75	0.6
2	*Mi-BrHM1-C2-Q1*	63.1	17.3	34.0	HM1	61–67	1.4
8	*Mi-BrHM1-C8-Q2*	41.9	8.4	13.7	B1091	41–56	1.0
9	*Mi-BrHM1-C9-Q4*	4.2	2.6	4.1	HM1	4–22	0.6
Summed % variance explained by multi-QTL model = 55.5%							
(SFF× HM2)							
2	*Mi-SFFHM2-C2-Q3*	42.6	2.8	8.0	HM2	4–66	1.1
4	*Mi-SFFHM2-C4-Q1*	33.3	4.6	13.4	SFF	15–57	1.0
8	*Mi-SFFHM2-C8-Q2*	41.5	3.2	9.2	SFF	27–59	0.8
Summed % variance explained by multi-QTL model = 34.8%							
(HM3)							
1	*Mi-HM3-C1-Q3*	34.8	4.0	4.3	HM3	23–65	0.4
8	*Mi-HM3-C8-Q2*	41.9	13.5	15.8	HM3	41–44	0.9
9	*Mi-HM3-C9-Q1*	9.6	14.9	17.7	HM3	4–13	0.1
Summed % variance explained by multi-QTL model = 35.7%							

[a]Percentage of variation explained
[b]1.5 LOD support interval (cM)
[c]Half phenotypic difference between means of resistant and susceptible homozygous genotypes
(revised from Parsons et al. (2015) *Meloidogyne incognita* nematode resistance QTLs in carrot. Mol Breeding 35:114)

evaluations made in one-week old plants (Yau et al. 2005). Boiteux et al. (2000) mapped the *Mj-1* gene that confers resistance to *Meloidogyne javanica* root-knot nematodes, and Boiteux et al. (2004) successfully identified homozygous resistant plants in breeding populations.

6.9 Candidate Genes

6.9.1 A Candidate Gene For Root Shape

The cultivated carrot storage root is typically much wider than the taproot of wild carrots. In the evaluation of a collection of wild and cultivated carrots, a polymorphic indel on chromosome 2 was associated with root diameter and referred to as

cult. Using a mapping population developed from a cross between wild and cultivated carrots, root diameter segregated and Macko-Podgorni et al. (2017) identified *DcAHLc1* as a candidate for *cult.* The genomic region that includes *cult* was among those identified as differentiating wild and domesticated carrots in a GWAS study (Ellison et al. 2018).

6.9.2 Genes for Pigments and Color

Three genes controlling the accumulation and distribution of orange and yellow carotenoids in the carrot storage root, *Y*, Y_2, and *Or*, have been mapped in segregating populations and candidate genes identified for *Y* and *Or*. The *Y* candidate is an interesting homolog of the *Arabidopsis thaliana* gene *PSEUDO-ETIOLATION IN LIGHT*, responsible for the regulation of photomorphogenesis. Two frameshift mutations identified turn off the constitutive repression of genes downstream that usually require exposure to light to trigger plastid biogenesis (Iorizzo et al. 2016). The Y_2 and *Or* genes described above both influence plastid development. *Or* was identified in GWAS, as described above. While a definite candidate for Y_2 has not been identified, a relatively short list including transcription factors and genes involved in light signaling and carbon flux are among them (Ellison et al. 2017).

The carotene hydroxylase gene is the candidate for controlling the relatively high amount of α-carotene in carrot roots. In transgenic experiments, Arango et al. (2014) overexpressed carotene hydroxylase *CYP97A3* in orange carrots and observed that the content of α-carotene in leaves and roots was several-fold higher than in control plants. Transgenic experiments involving overexpression of *CYP97A3* lowered α-carotene content of leaves and carrots.

Three genes, *P1, P2,* and *P3*, control anthocyanin accumulation in purple carrots. *P3* controls root and petiole pigmentation and a MYB, *DcMYB7*, was identified as a candidate (Iorizzo et al. 2019b). *DcMYB7* is in a cluster of MYB genes and its identification as the candidate is based on fine mapping plus transcriptome analysis.

6.9.3 A Candidate for Sugar Type

Most carrots store a mixture of glucose and fructose but a single gene mutation, *Rs,* was discovered to condition storage roots to primarily accumulate sucrose (Freeman and Simon 1983). Yau and Simon (2003) determined that a 2.5 kb insert in the acid-soluble invertase II gene was associated with *Rs* so that roots of carrots homozygous *rs/rs* accumulate sucrose.

6.10 Genomics-Assisted Breeding and Genome Editing for CS Traits

Systematic investigations on the genetics of abiotic stress tolerance in carrot are of high significance, as they are essential for the development of new cultivars better adapted to the changing environmental conditions imposed by global warming. It can be obtained by exploring the existing genetic diversity both in the cultivated gene pool and in the wild crop relatives. For instance, wild *D. carota* and carrot landraces subspecies might be a source for increased tolerance to salinity (Kasiri et al. 2013). Kiełkowska et al. (2019) showed that increased tolerance to salinity in some Iranian landraces and their progeny was related to higher anthocyanin accumulation in petioles and increased trichome formation on leaves and petioles. While carrots have been widely cultivated in temperate climatic zones, efforts have been undertaken to breed for varieties that could be cultivated in warmer regions. In Brazil, breeding of carrot cultivars suitable for production in the subtropical climate using well-adapted local landraces of the European origin was successful. The open-pollinated cultivar "Brasilia" and its derivatives constitute the major fraction of carrot production there (Simon et al. 2008). Elucidation of major genetic determinants of adaptation to abiotic stresses and incorporation of molecular tools in breeding would certainly shorten the time required for developing and selecting plant materials showing desired characteristics, which could subsequently be introduced for production in regions suffering from malnutrition and vitamin A deficiency, supporting previous efforts implementing conventional selection methods. Application of molecular techniques (e.g., marker-assisted backcrossing) might also support more efficient transfer of abiotic stress tolerances present in the wild *D. carota* gene pool.

Genetic modifications might be another method of choice, depending on the public acceptance of genetic transformation and novel, more precise techniques of gene editing. Abiotic stresses can be applied postharvest, in order to increase synthesis of valuable biologically active secondary metabolites. Carrot is highly amenable for genome engineering, using both transgenesis (Baranski 2008) and CRISPR/Cas9 genome editing (Klimek-Chodacka et al. 2018; Baranski and Lukasiewicz 2019; Xu et al. 2019). The latter technology has appeared very recently as a new possibility, and has not yet been implemented as a tool to modify the reaction of plants to abiotic stresses. However, genetic transformation has been used to improve carrot tolerance and several reports on the expression of heterologous stress-related genes in carrot have been published. Transgenic carrot plants carrying a gene coding for betaine aldehyde dehydrogenase (BADH) showed highly increased betaine content and significantly improved tolerance to salt stress (Kumar et al. 2004). Carrot transformation with mammalian 6-phosphofructo-2-kinase/fructose2,6-bisphosphatase (6-PF-2-K/Fru2,6-P2ase) gene resulted in highly increased levels of fructose 2,6-bisphosphate (Fru-2,6-P2) in roots of the transgenic plants. Under drought and cold stress, it allowed the mobilization of energy reserves by gluconeogenesis (Kovács et al. 2006). Attempts have been undertaken to use genetic engineering, which allows the introduction of specific genes, closely related to the production of compounds that

give the plant advantage in stress conditions. For this purpose, different approaches have been used: (a) the introduction of genes involved in ion and water uptake or ions transport; (b) genes encoding osmolytes, such as glycine, mannitol, or proline; (c) genes encoding transcription factors like MAPK, DREB1, and others (Parmar et al. 2017).

Among pathogenesis-related protein family PR-5, there are osmotins that have been isolated for the first time from cell cultures of tobacco (Singh et al. 1985). These proteins are usually located in the electron-dense inclusion bodies in the vacuoles. Their synthesis is regulated by various hormonal and environmental factors, including abiotic stress (salinity or desiccation). Osmotins are presumed to protect cell membranes causing membrane permeability during stress, resulting in increased tolerance in transgenic tobacco (Barthakur et al. 2001), wheat (Noori and Sokhansanj 2008), or pepper (Subramanyam et al. 2010). Callus formed from carrot hypocotyl explants was transformed with a truncated tobacco osmotin gene lacking the sequence encoding a 20-aminoacid C-terminal end (Annon et al. 2014). Removal of the C-terminal end fragment results in extracellular secretion of the protein. Transgenic lines with the overexpression of tobacco osmotin conferred tolerance to drought stress in carrot plants and exhibited faster and fuller recovery than control plants after drought treatment. Transformed plants had also higher water content, less ion leakage, lower level of lipid peroxidation, and higher relative water content. Tolerance to drought as desiccation was also the subject of research by Shiota and Kamada (2000). As a result of the research, non-embryogenic carrot cells with a high expression of C-ABI3 gene, a carrot homolog of the VPI/ABI3 gene, were obtained. This enabled tolerance of desiccation upon ABA treatment.

In plants grown in saline soil, an increased accumulation of osmoprotective compounds (glycine betaine (Gly betaine) and β-alanine betaine) is often observed, which allow plant cells to maintain homeostasis. The synthesis of Gly betaine in plants involves choline monooxygenase and BADH, which are localized in chloroplasts. Kumar et al. (2004) performed successful engineering of the carrot chloroplast genome with the vector pDD-*Dc-aadA*/*badh* by homologous recombination in the 16S-23S spacer region. Researchers observed an increase in tolerance to salinity in both cell suspension cultures and plants. Transformed cells were able to survive higher NaCl concentrations. The activity of BADH enzyme was eightfold higher in the presence of 100 mM of NaCl, and 50 times more betaine was accumulated in the transformed cells, as compared to the wild type. Transgenic plants tolerated salinity at 400 mM of NaCl, whereas non-transformed plants exhibited severe growth reduction at 200 mM of NaCl. Also, Han and Hwang (2003) performed genetic transformation of carrot to enhance salt tolerance. Researchers introduced pyrroline-5-carboxylate synthetase (P5CS) gene from moth bean which is a key gene in regulation of proline biosynthesis. Proline is known as an osmoprotectant that is accumulated in large quantities in response to environmental stresses (Ashraf and Foolad 2007). Proline is responsible for the stabilization of sub-cellular structures (e.g., membranes and proteins), scavenging free radicals, and regulating the cellular redox potential. The P5CS gene under control of P35S promoter was transferred to carrot cells via *Agrobacterium* genetic transformation. The transgenic cell lines showed six times increased

relative growth following treatment with 250 mM NaCl, as compared to wild type cells. Also, a significant, up to sixfold, increase of proline content in transgenic cells was observed.

Recent years have brought a new tool that allows even more precise modification of plant DNA: Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated protein-9 nuclease (Cas9). In this system, the Cas9 protein derived from *Streptococcus pyogenes* is engineered to target specific DNA based on Watson–Crick base complementary paring and to create double-stranded breaks. An important role is also played by the, usually 3-nucleotide, protospacer adjacent motif (PAM) located directly at the recognized DNA sequence (Mushtaq et al. 2018). The resulting breaks in the DNA are then repaired by homologous recombination (HDR) or nonhomologous end joining (NHEJ), which is often accompanied by point mutations. The CRISPR/Cas9 system was developed in model plants such as *Arabidopsis* (Jiang et al. 2013; Mao et al. 2013), rice (Feng et al. 2013), and tobacco (Li et al. 2013), but also carrot (Klimek-Chodacka et al. 2018). It has been successfully used for genome editing endogenous genes of many crop plants, mainly causing phenotypic changes such as change in the content of biochemical compounds, development of parthenocarpy, and increase in tolerance to diseases (Mushtaq et al. 2018). CRISPR technology has also been successfully used to obtain plants tolerating abiotic stresses. Osakabe and Osakabe (2017) focused on the *OPEN STOMATA 2* (*OST2*) (*AHA1*) gene encoding a plasma membrane H^+-ATPase in the stomatal response in Arabidopsis. The mutation contributed to faster stomatal closing during abiotic stress, resulting in significantly reduced water loss rates in leaves of engineered plants. CRISPR technology has also been used for editing the maize *ARGOS8* gene, a negative regulator of ethylene responses (Shi et al. 2017). It has already been demonstrated that overexpression of the *ARGOS8* gene resulted in increased grain yield under drought conditions but has no effect on yield under optimal conditions (Shi et al. 2015). The CRISPR-edited variants of the gene also enabled its overexpression and the increase of yield of drought-stressed plants.

Currently, it seems that the CRISPR technology will allow us to achieve significant progress and allow for a significant advantage of plants over abiotic stresses. Plant response to stress factors is very complex, including numerous interactions between signaling, regulatory, and metabolic pathways (Jain 2015). Often, these genes are represented by multi-gene families with functional redundancy, which are also associated with duplications present in the genome. The CRISPR system, thanks to its simplicity, is an ideal tool for simultaneous editing of a number of genes.

6.11 Bioinformatic Tools

The first carrot plastid genome (Ruhlman et al. 2006), several additional plastid and mitochondrial genomes, and two draft nuclear genomes have been published. The two available nuclear genomes include an assembly of 371.6 Mb at CarrotDB corresponding to 32 × coverage (Xu et al. 2014), and an assembly of 421.5 Mb

corresponding to 186 × coverage (Iorizzo et al. 2016). A dedicated, comprehensive bioinformatics platform for carrot and other Apiaceae called CarrotOmics is being developed (Bostan et al. 2019). Transcriptome data, linkage maps based on all marker systems, phenotypic data, and other "omics" data will be included at CarrotOmics.

6.12 Future Perspectives

Carrot production has risen in recent decades with an especially large increase in Asia. With anticipated challenges from heat, drought, and salinity arising from climate change in as soon as the next few decades, combined with much of the newer carrot production being realized in warmer climatic regions of the world, the urgency for dedicating a significant effort to improved abiotic stress tolerance by carrot breeders and other scientists involved in applied agricultural research is critical. The broad range of genetic diversity in carrot germplasm provides a strong foundation for undertaking this important effort, and the growing availability of genome-assisted breeding tools will make that task more efficient. The significant nutritional contribution that carrot can deliver to warm, dry regions of the developing world as a sustainable vitamin A source with a relatively long postharvest storage shelf life provides an additional incentive for developing nutritious CS carrots.

References

Acosta-Motos JR, Ortuño M, Bernal-Vicente A, Diaz-Vivancos P, Sanchez-Blanco M, Hernandez J (2017) Plant responses to salt stress: adaptive mechanisms. Agronomy 7:18. https://doi.org/10.3390/agronomy7010018

AhnY-J, SongN (2012) A cytosolic heat shock protein expressed in carrot (*Daucus carota* L.) enhances cell viability under oxidative and osmotic stress conditions. HortScience47:143–148

Alessandro MS, Galmarini CR (2007) Inheritance of vernalization requirement in carrot. J Amer Soc Hort Sci 132:525–529

Alessandro MS, Galmarini CR, Iorizzo M, Simon PW (2013) Molecular mapping of vernalization requirementand fertility restoration genes in carrot. Theor Appl Genet 126:415–423

Ali A, Matthews WC, Cavagnaro PF, Iorizzo M, Roberts PA, Simon PW (2013) Inheritance and mapping of *Mj-2*, a new source of root-knot nematode (*Meloidogyne javanica*) resistance in carrot. J Hered105:288–291

Algarra M, Fernandes A, Mateus N, de Freitas V, Esteves da Silva JCG, Casado J (2014) Anthocyanin profile and antioxidant capacity of black carrots (*Daucus carota* L. ssp. *sativus* var. *atrorubens* Alef.) from Cuevas Bajas. Spain. J Food Compos Anal 33:71–76. https://doi.org/10.1016/j.jfca.2013.11.005

Allender C (2019) Genetic resources for carrot improvement. In: Simon PW, Iorizzo M, Grzebelus D, Baranski R (eds) The Carrot Genome. Springer, Cham, Switzerland, pp 93–100

Almansouri M, Kinet J-M, Lutts S (2001) Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* desf.). Plant Soil 231:243–254. https://doi.org/10.1023/A:1010378409663

Amirsadeghi S, McDonald A, Vanlerberghe G (2007) A glucocorticoid-inducible gene expression system can cause growth defects in tobacco. Planta 226:453–463. https://doi.org/10.1007/s00425-007-0495-1

Annon A, Rathore K, Crosby K (2014) Overexpression of a tobacco osmotin gene in carrot (*Daucus carota* L.) enhances drought tolerance. Vitro Cell Dev Biol—Plant 50:299–306. https://doi.org/10.1007/s11627-013-9590-0

Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 55:373–399. https://doi.org/10.1146/annurev.arplant.55.031903.141701

Arango J, Jourdan M, Geoffriau E et al (2014) Carotenehydroxylase activity determines the levels of bothalpha-carotene and total carotenoids in orange carrots. Plant Cell 26:2223–2233

Arscott SA, Tanumihardjo SA (2010) Carrots of many colors provide basic nutrition and bioavailable phytochemicals acting as a functional food. Compr Rev Food Sci Food Saf 9:223–239

Asada K (2006) Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol 141:391. https://doi.org/10.1104/pp.106.082040

Ashraf M, Foolad MR (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ Exp Bot 59:206–216. https://doi.org/10.1016/j.envexpbot.2005.12.006

Bach IC, Olesen A, Simon PW (2002) PCR-basedmarkers to differentiate the mitochondrial genome ofpetaloid and male fertile carrot (*Daucus carota* L.). Euphytica 127:353–365

Bado S, Forster B, Ghanim A, Jankowicz-Cieslak J, Berthold J, Luxiang L (2016) Protocols for pre-field screening of mutants for salt tolerance in rice, wheat and barley. Springer Internat Pub

Banasiak Ł, Wojewódzka A, Baczyński J, Reduron J-P, Piwczyński M, Kurzyna-Młynik R, Gutaker R, Czarnocka-Cieciura A, Kosmala-Grzechnik S, Spalik K (2016) Phylogeny of Apiaceae subtribe Daucinae and the taxonomic delineation of its genera. Taxon 65:563–585

Banga O (1957a) Origin of the European cultivated carrot. Euphytica 6:54–63

Banga O (1957b) The development of the original European carrot material. Euphytica 6:64–76

Banga O (1963) Main Types of the Western Carotene Carrot and Their Origin. W.E.J, Tjeenk Willink, Zwolle, The Netherlands

Bano S, Ashraf M, Akram N (2014) Salt stress regulates enzymatic and nonenzymatic antioxidative defense system in the edible part of carrot (*Daucus carota* L.). Plant-Environ Interact 9:324–329. https://doi.org/10.1080/17429145.2013.832426

Baranski R (2008) Genetic transformation of carrot (*Daucus carota*) and other Apiaceae species. Transgen Plant J 2:18–38

Baranski R, Lukasiewicz A (2019) Genetic engineering of carrot. In: Simon PW, Iorizzo M, Grzebelus D, Baranski R (eds) The Carrot Genome. Springer, Cham, Switzerland, pp 149–186

Barnes WC (1936) Effects of some environmental factors on growth and color of carrots. NY Agri Exper Stn Ithaca Memoirs 186:1–36

Barthakur S, Babu V, Bansa KC (2001) Over-expression of osmotin induces proline accumulation and confers tolerance to osmotic stress in transgenic tobacco. J Plant BiochemBiotechnol 10:31–37. https://doi.org/10.1007/bf03263103

Baxter I (2009) Ionomics: studying the social network of mineral nutrients. Curr Opin Plant Biol 12:381–386. https://doi.org/10.1016/j.pbi.2009.05.002

Bernstein L, Ayers A (1953) Salt tolerance of five varieties of carrots. J Amer Soc Hort Sci 61:360–366

Berry J, Bjorkman O (1980) Photosynthetic response and adaptation to temperature in higher plants. Annu Rev 31:491–543. https://doi.org/10.1146/annurev.pp.31.060180.002423

Boiteux LS, Belter JG, Roberts PA, Simon PW (2000) RAPD linkage map of the genomic region encompassing the root-knot nematode (*Meloidogyne javanica*) resistance locus in carrot. Theor Appl Genet 100:439–446

Boiteux LS, Hyman JR, Bach IC, Fonseca MEN, Matthews WC, Roberts PA, Simon PW (2004) Employment of flanking codominant STS markers to estimate allelic substitution effects of a nematode resistance locus in carrot. Euphytica 136:37–44

Bolton A, Nijabat A, Mahmood-ur-Rehman M, Naveed NH, Mannan ATMM, Ali A, Rahim MA, Simon PW (2019) Variation for heat tolerance during seed germination in diverse carrot [*Daucus carota* (L.)] germplasm. HortScience 54:1470–1476. https://doi.org/10.21273/HORTSCI14144-19

Bolton A, Simon P (2019) Variation for salinity tolerance during seed germination in diverse carrot [*Daucus carota* (L.)] germplasm. HortScience 54:38–44. https://doi.org/10.21273/HORTSCI13333-18

Borner T, Linke B, Nothnagel T, Scheike R et al (1995) Inheritance of nuclear and cytoplasmic factors affectingmale sterility in *Daucus carota*. Adv Plant Breed 18:111–122

Bostan H, Senalik D, Simon PW, Iorizzo M (2019) Carrot genetics, omics and breeding toolboxes. In: Simon PW, Iorizzo M, Grzebelus D, Baranski R (eds) The Carrot Genome. Springer, Cham, Switzerland, pp 225–246

Bowen J, Lay-Yee M, Plummer KIM, Ferguson IAN (2002) The heat shock response is involved in thermotolerance in suspension-cultured apple fruit cells. J Plant Physiol 159:599–606 https://doi.org/10.1078/0176-1617-0752

Boyer JS (1982) Plant productivity and environment. Science 218:443–448. https://doi.org/10.1126/science.218.4571.443

Bradeen J, Simon P (1998) Conversion of an AFLPfragment linked to the carrot Y2 locus to a simple, codominant, PCR-based marker form. Theor ApplGenet 97:960–967

Bradeen JM, Simon PW (2007) Carrot. In: Kole C (ed) GenomeMapping and Molecular Breeding in Plants, vol 5. Vegetables. Springer, Heidelberg, pp 161–184

Bradeen JM, Bach IC, Briard M, Le Clerc V, Grzebelus D, Senalik DA Simon PW (2002) Molecular diversity analysis of cultivated carrot (*Daucus carota* L.) and wild *Daucus* populations reveals a genetically nonstructured composition. J Amer Soc Hort Sci 127:383–391

Bray E, Bailey-Serres J, Weretilnyk E (2000) Responses to abiotic stresses. Biochemistry and Molecular Biology of Plants. American Society of Plant Physiologists, Rockville, MD, pp 1158–1249

Broussard MA, Mas F, Howlett B, Pattemore D, Tylianakis JM (2017) Possible mechanisms of pollination failure in hybrid carrot seed and implications for industry in a changing climate. PLoS ONE 12:e0180215. https://doi.org/10.1371/journal.pone.0180215

Budahn H, Barański R, Grzebelus D, Kiełkowska et al(2014) Mapping genes governing flower architectureand pollen development in a double mutant populationof carrot. Front Plant Sci 5:504

Camejo D, Jiménez A, Alarcón JJ, Torres W, María Gómez J, Sevilla F (2006) Changes in photosynthetic parameters and antioxidant activities following heat-shock treatment in tomato plants.Functional Plant Biol 33:177–187 https://doi.org/10.1071/fp05067

Campos MD, Cardoso HG, Linke B, Costa JH, De Melo DF, Justo L, Frederico AMF, Arnholdt-Schmitt B (2009) Differential expression and co-regulation of carrot AOX genes (*Daucus carota*). Physiol Plant 137 doi:10.1111/j.1399-3054.2009.01282.x

Campos MD, Nogales A, Cardoso HG, Kumar SR, Nobre T, Sathishkumar R, Arnholdt-Schmitt B (2016) Stress-induced accumulation of *DcAOX1* and *DcAOX2a* transcripts coincides with critical time point for structural biomass prediction in carrot primary cultures (*Daucus carota* L.). Front Genet 7 https://doi.org/10.3389/fgene.2016.00001

Carillo P, Annunziata M, Pontecorvo G, Fuggi A, Woodrow P (2011) Salinity stress and salt tolerance. In: Shanker A, Venkateswaralu B (eds)Abiotic Stress in Plants—Mechanisms and Adaptations, 1st edn. INTECH, pp 21–38

Castaneda-Alvarez NP, Khoury CK, Achicanoy HA, Bernau V, Dempewolf H, Eastwood RJ, Guarino L, Harker RH, Jarvis A, Maxted N, Muller JV, Ramirez-Villegas J, Sosa CC, Struik PC, Vincent H, Toll J (2016) Global conservation priorities for crop wild relatives. Nat Plants 2:16022

Cavagnaro P, Iorizzo M (2019) Carrot anthocyanin diversity, genetics and genomics. In: Simon PW, Iorizzo M, Grzebelus D, Baranski R (eds) The Carrot Genome. Springer, Cham, Switzerland, pp 261–278

Cavagnaro PF, Iorizzo M, Yildiz M, Senalik D, Parsons J, Ellison S, Simon PW (2014) A gene-derived SNP-based high resolution linkage map of carrot including the location of QTL conditioning root and leaf anthocyanin pigmentation. BMC Genom 15:1118

Choudhary R, Saroha A, Swarnkar P (2012) Effect of abscisic acid and hydrogen peroxide on antioxidant enzymes in *Syzygium cumini* plant. J Food Sci Technol 49:649–652. https://doi.org/10.1007/s13197-011-0464-3

Collier R, Finch S (2009) A review of research to address carrot fly (*Psila rosae*) control in the UK. EPPO Bull 39:121–127

Costa JH, Cardoso HG, Campos MD, Zavattieri A, Frederico AM, Fernandes de Melo D, Arnholdt-Schmitt B (2009) *Daucus carota* L.—An old model for cell reprogramming gains new importance through a novel expansion pattern of alternative oxidase (*AOX*) genes. Plant Physiol Biochem 47:753–759. https://doi.org/10.1016/j.plaphy.2009.03.011%5bCorrigendum to "*Daucus carota*L.- an old model for cell reprogramming gains new importance through a novel expansion patternof alternative oxidase (*AOX*) genes. Plant Physiol Biochem 85:114." doi:https://doi.org/10.1016/j.plaphy.2014.11.013]

Davenport R, James RA, Zakrisson-Plogander A, Tester M, Munns R (2005) Control of sodium transport in Durum wheat. Plant Physiol 137:807. https://doi.org/10.1104/pp.104.057307

DeRose-Wilson L, Gaut B (2011) Mapping salinity tolerance during *Arabidopsis thaliana* germination and seedling growth. PLoS One 6 doi:10.1371/journal.pone.0022832

Di Mascio P, Kaiser S, Sies H (1989) Lycopene as the most efficient biological carotenoid singlet oxygen quencher. Arch Biochem Biophys 274:532–538. https://doi.org/10.1016/0003-9861(89)90467-0

du Toit LJ, Le Clerc V, Briard M (2019) Genetics and genomics of carrot biotic stress. In: Simon PW, Iorizzo M, Grzebelus D, Baranski R (eds) The Carrot Genome. Springer, Cham, Switzerland, pp 317–362

Duke JA (1992) Handbook of Phytochemical Constituents of GRAS Herbs and Other Economic Plants. CRC Press, Boca Raton, FL

Ellison S, Senalik D, Bostan H, Iorizzo M, Simon P (2017) Fine mapping, transcriptome analysis, andmarker development for *Y2*, the gene that conditionsbeta-carotene accumulation in carrot (*Daucus carota*L.). G3: Genes Genom Genet7:2665–2675. https://doi.org/10.1534/g3.117.043067

Ellison S, Luby C, Corak K, Coe K et al (2018) Association analysis reveals the importance of the *Or* gene in carrot (*Daucus carota* L.) carotenoid presence and domestication. Genetics 210:1–12

Eryılmaz F (2006) The relationships between salt stress and anthocyanin content in higher plants. Biotechnol & Biotechnol Equip 20:47–52. https://doi.org/10.1080/13102818.2006.10817303

Essemine J, Ammar S, Bouzid S (2010) Impact of heat stress on germination and growth in higher plants: physiological, biochemical and molecular repercussions and mechanisms of defence. J Biol Sci 10:565–572. https://doi.org/10.3923/jbs.2010.565.572

Fahad S, Bajwa AA, Nazir U, Anjum SA, Farooq A, Zohaib A, Sadia S, Nasim W, Adkins S, Saud S, Ihsan MZ, Alharby H, Wu C, Wang D, Huang J (2017) Crop production under drought and heat stress: plant responses and management options. Front Plant Sci 8:1147. https://doi.org/10.3389/fpls.2017.01147

FAO (2019) www.fao.org/statistics

Feller U, Crafts-Brandner SJ, Salvucci ME (1998) Moderately high temperatures inhibit ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activase-mediated activation of Rubisco. Plant Physiol 116:539. https://doi.org/10.1104/pp.116.2.539

Feng Z, Zhang B, Ding W, Liu X, Yang D-L, Wei P, Cao F, Zhu S, Zhang F, Mao Y, Zhu JK (2013) Efficient genome editing in plants using a CRISPR/Cas system. Cell Res 23:1229–1232. https://doi.org/10.1038/cr.2013.114

Flowers TJ (2004) Improving crop salt tolerance. J Exp Bot 55:307–319. https://doi.org/10.1093/jxb/erh003

Freeman RE, Simon PW (1983) Evidence for simple genetic control of sugar type in carrot (*Daucus carota* L.). J Amer Soc Hort Sci 108:50–54

Fuentes P, Pizarro L, Moreno JC, Handford M, Rodriguez-Concepcion M, Stange C (2012) Light-dependent changes in plastid differentiation influence carotenoid gene expression and accumulation in carrot roots. Plant Mol Biol 79:47–59. https://doi.org/10.1007/s11103-012-9893-2

Garciarrubio A, Legaria J, Covarrubias A (1997) Abscisic acid inhibits germination of mature *Arabidopsis* seeds by limiting the availability of energy and nutrients. Planta 203:182–187. https://doi.org/10.1007/s004250050180

Gibberd MR, Turner NC, Storey R (2002) Influence of saline irrigation on growth, ion accumulation and partitioning, and leaf gas exchange of carrot (*Daucus carota* L.). Ann Bot 90:715–724. https://doi.org/10.1093/aob/mcf253

Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48:909–930. https://doi.org/10.1016/j.plaphy.2010.08.016

Gong M, Chen S, Song Y, Li Z (1997) Effect of calcium and calmodulin on intrinsic heat tolerance in relation to antioxidant systems in maize seedlings. Aust J Plant Physiol 24:371–379. https://doi.org/10.1071/PP96118

Graßmann J (2005) Terpenoids as Plant Antioxidants. In: Litwack G (ed) Vitamins andHormones. Academic Press, New Yorkpp, pp 505–535

Greer D, Weedon M (2012) Modelling photosynthetic responses to temperature of grapevine (*Vitis vinifera* cv. 'Semillon') leaves on vines grown in a hot climate. Plant, Cell Environ 35:1050–1064. https://doi.org/10.1111/j.1365-3040.2011.02471.x

Gregorio G, Senadhira D, Mendoza R (1997) Screening Rice for Salinity Tolerance. IRRI, Manila, Philippines

Groves RL, Clements JR, Bradford BZ (2019) Carrot diseases resulting from phytoplasmas and viruses. In: Geoffriau E, Simon PW (eds) Carrot and Other Cultivated Apiaceae, CABI, Oxford, UK, in press

Grzebelus D (2019) Genetics and genomics of carrot abiotic stress. In: Simon PW, Iorizzo M, Grzebelus D, Baranski R (eds) The Carrot Genome. Springer, Switzerland, pp 363–372

Guajardo E, Correa J, Contreras-Porcia L (2016) Role of abscisic acid (ABA) in activating antioxidant tolerance responses to desiccation stress in intertidal seaweed species. Planta 243:767–781. https://doi.org/10.1007/s00425-015-2438-6

Han KH, Hwang CH (2003) Salt tolerance enhanced by transformation of a P5CS gene in carrot. J Plant Biotechnol 5:157–161

Hasanuzzaman M, Nahar K, Alam MM, Roychowdhury R, Fujita M (2013) Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. Intl J Mol Sci 14:9643–9684. https://doi.org/10.3390/ijms14059643

Hooper PL, Hooper PL, Tytell M, Vígh L (2010) Xenohormesis: health benefits from an eon of plant stress response evolution. Cell Stress Chaperones 15:761–770. https://doi.org/10.1007/s12192-010-0206-x

Huang B, Xu C (2008) Identification and characterization of proteins associated with plant tolerance to heat stress. J Integr Plant Biol 50:1230–1237. https://doi.org/10.1111/j.1744-7909.2008.00735.x

Huang Y, Li M-Y, Wang F, Xu Z-S, Huang W, Wang G-L, Ma J, Xiong A-S (2015) Heat shock factors in carrot: genome-wide identification, classification, and expression profiles response to abiotic stress. Mol Biol Rep 42:893–905. https://doi.org/10.1007/s11033-014-3826-x

Hussain HA, Hussain S, Khaliq A, Ashraf U, Anjum SA, Men S, Wang L (2018) Chilling and drought stresses in crop plants: implications, cross talk, and potential management opportunities. Front Plant Sci 9:393. https://doi.org/10.3389/fpls.2018.00393

Iorizzo M, Senalik DA, Ellison SL, Grzebelus D, Cavagnaro PF, Allender C, Brunet J, Spooner DM, Van Deynze A, Simon PW (2013) Genetic structure and domestication of carrot (*Daucus carota* L. subsp. *sativus* L.) (Apiaceae). Amer J Bot 100:930–938

Iorizzo M, Ellison S, Senalik D, Zeng P, Satapoomin P, Huang J, Bowman M, Iovene M, Sanseverino W, Cavagnaro P, Yildiz M, Macko-Podgórni A, Moranska E, Grzebelus E, Grzebelus D, Ashrafi

H, Zheng Z, Cheng S, Spooner D, Van Deynze A, Simon P (2016) A high-quality carrot genome assembly provides new insights into carotenoid accumulation and asterid genome evolution. Nat Genet 48:657–666. https://doi.org/10.1038/ng.3565

Iorizzo M, Ellison S, Pottorff M, Cavagnaro P (2019a) Carrot molecular genetics and mapping. In: Simon PW, Iorizzo M, Grzebelus D, Baranski R (eds) The Carrot Genome. Springer Nature, Cham, Switzerland, pp 101–118

Iorizzo M, Cavagnaro P, Bostan A, Zhao Y, Zhang J, Simon PW (2019b) A cluster of MYB transcription factors regulates anthocyanin biosynthesis in carrot (*Daucus carota* L.) root and petiole. Front Plant Sci 9:1927

Jain M (2015) Function genomics of abiotic stress tolerance in plants: a CRISPR approach. Front Plant Sci 6. https://doi.org/10.3389/fpls.2015.00375

Jiang W, Zhou H, Bi H, Fromm M, Yang B, Weeks DP (2013) Demonstration of CRISPR/Cas9/sgRNA-mediated targeted gene modification in *Arabidopsis*, tobacco, sorghum and rice Nucl Acids Res 41:e188–e188. https://doi.org/10.1093/nar/gkt780

Jourdan M, Gagne S, Dubois-Laurent C et al (2015) Carotenoid content and root color of cultivated carrot: a candidate-gene association study using an originalbroad unstructured population. PLoS ONE 10:e0116674

Jung YC, Lee HJ, Yum SS, Soh WY, Cho DY, Auh CK, Lee TK, Soh HC, Kim YS, Lee SC (2005) Drought-inducible—but ABA-independent—thaumatin-like protein from carrot (*Daucus carota* L.). Plant Cell Rep 24:366–373

Just BJ, Santos CA, Yandell BS, Simon PW (2009) Major QTL for carrot color are positionally associatedwith carotenoid biosynthetic genes and interactepistatically in a domesticated x wild carrot cross.Theor Appl Genet 119:1155–1169

Kahouli B, Borgi Z, Hannachi C (2014) Effect of sodium chloride on the germination of the seeds of a collection of carrot accessions (*Daucus carota* L.) cultivated in the region of Sidi Bouzid. J Stress Physiol Biochem 10:28–36

Kasiri MR, Hassandokht MR, Kashi A, Shahi-Gharahlar A (2013) Evaluation of genetic diversity in Iranian yellow carrot accessions (*Daucus carota* var. *sativus*), an exposed to extinction rooty vegetable, using morphological characters. Intl J Agri Crop Sci 6:151–156

Keilwagen J, Lehnert H, Berner T, Budahn H, Nothnagel T, Ulrich D, Dunemann F (2017) The terpene synthase gene family of carrot (*Daucus carota* L.): Identification of QTLs and candidate genes associated with terpenoid volatile compounds. Front Plant Sci 8:1930. https://doi.org/10.3389/fpls.2017.01930

Kiełkowska A, Grzebelus E, Lis-Krzyścin A, Maćkowska K (2019) In vitro selection in protoplast cultures of the carrot (*Daucus carota* L.) and evaluation of the response of regenerants to soil salinity. Plant Cell Tiss Organ Cult 137:379–395

Klimek-Chodacka M, Oleszkiewicz T, Lowder LG, Qi Y, Baranski R (2018) Efficient CRISPR/Cas9-based genome editing in carrot cells. Plant Cell Rep 37:575–586. https://doi.org/10.1007/s00299-018-2252-2

Kovács G, Sorvari S, Scott P, Toldi O (2006) Pyrophosphate: fructose 6-phosphate 1-phosphotransferase operates in net gluconeogenic direction in taproots of cold and drought stressed carrot plants. Acta Biol 50:25–30. https://doi.org/10.1556/AAgr.55.2007.1.8

Kovinich N, Kayanja G, Chanoca A, Otegui M, Grotewold E (2015) Abiotic stresses induce different localizations of anthocyanins in *Arabidopsis*. Plant Signal Behav 10. https://doi.org/10.1080/15592324.2015.1027850

Krishnamurthy A, Rathinasabapathi B (2013) Oxidative stress tolerance in plants: novel interplay between auxin and reactive oxygen species signaling. Plant Signal Behav 8. https://doi.org/10.4161/psb.25761

Kumar S, Dhingra A, Daniell H (2004) Plastid-expressed betaine aldehyde dehydrogenase gene in carrot cultured cells, roots, and leaves confers enhanced salt tolerance. Plant Physiol 136:2843–2854. https://doi.org/10.1104/pp.104.045187

Landi M, Tattini M, Gould KS (2015) Multiple functional roles of anthocyanins in plant-environment interactions. Funct Roles Second Metab Plant-Environ Interact 119:4–17. https://doi.org/10.1016/j.envexpbot.2015.05.012

Landjeva S, Neumann K, Lohwasser U, Börner A (2008) Molecular mapping of genomic regions associated with wheat seedling growth under osmotic stress. Biol Plant 52:259–266. https://doi.org/10.1007/s10535-008-0056-x

Larkindale J, Knight MR (2002) Protection against heat stress-induced oxidative damage in *Arabidopsis* involves calcium, abscisic acid, ethylene, and salicylic acid. Plant Physiol 128:682. https://doi.org/10.1104/pp.010320

Laufer B (1919) Sino-Iranica. Chicago, Field Museum of Natural Hist. Pub. 201; Anthropot Ser 15:451–454

Le Clerc V, Briard M (2019) Carrot disease management. In: Geoffriau E, Simon PW (eds) Carrot and other cultivated Apiaceae, CABI, Oxford, UK, in press

Le Clerc V, Pawelec A, Birolleau-Touchard C, Suel A, Briard M (2009) Genetic architecture of factors underlying partial resistance to Alternaria leaf blight in carrot. Theor Appl Genet 118:1251–1259

Le Clerc V, Marques S, Suel A, Huet S, Hamama L, Voisine L, Auperpin E, Jourdan M, Barrot L, Prieur R (2015) QTL mapping of carrot resistance to leaf blight with connected populations: stability across years and consequences for breeding. Theor Appl Genet 128:2177–2187

Li J-F, Norville JE, Aach J, McCormack M, Zhang D, Bush J, Church GM, Sheen J (2013) Multiplex and homologous recombination–mediated genome editing in *Arabidopsis* and *Nicotianabenthamiana* using guide RNA and Cas9. Nat Biotechnol 31:688–691. https://doi.org/10.1038/nbt.2654

Linke B, Alessandro MS, Galmarini C, Nothnagel T (2019) Carrot floral development and reproductive biology. In: Simon PW, Iorizzo M, Grzebelus D, Baranski R (eds) The Carrot Genome. Springer, Cham, Switzerland, pp 27–58

Maas E, Hoffman G (1977) Crop salt tolerance—current assessment. J Irrig Drain Div 103:115–134

Mackevic VI (1929) The carrot of Afghanistan. Bul Appl Bot Genet Plant Breeding 20:517–562

Macko-Podgórni A, Machaj G, Stelmach K, Senalik D et al (2017) Characterization of a genomic region under selection in cultivated carrot (*Daucus carota* subsp. *sativus*) reveals a candidate domestication gene. Front Plant Sci 8:12

Maestri E, Klueva N, Perrotta C, Gulli M, Nguyen H, Marmiroli N (2002) Molecular genetics of heat tolerance and heat shock proteins in cereals. Plant Mol Biol 48:667–681. https://doi.org/10.1023/A:1014826730024

Malik MK, Slovin JP, Hwang CH, Zimmerman JL (1999) Modified expression of a carrot small heat shock protein gene *Hsp17.7*, results in increased or decreased thermotolerance. Plant J 20:89–99

Mao Y, Zhang H, Xu N, Zhang B, Gou F, Zhu J-K (2013) Application of the CRISPR–Cas system for efficient genome engineering in plants. Mol Plant 6:2008–2011. https://doi.org/10.1093/mp/sst121

Mittler R, Blumwald E (2010) Genetic engineering for modern agriculture: challenges and perspectives. Annu Rev Plant Biol 61:443–462. https://doi.org/10.1146/annurev-arplant-042809-112116

Møller I, Jensen P, Hansson A (2007) Oxidative modifications to cellular components in plants. Annu Rev Plant Biol 58:459–481. https://doi.org/10.1146/annurev.arplant.58.032806.103946

Morales D, Rodríguez P, Dell'Amico J, Nicolás E, Torrecillas A, Sánchez-Blanco M (2003) High-temperature preconditioning and thermal shock imposition affects water relations, gas exchange and root hydraulic conductivity in tomato. Biol Plant 47:203. https://doi.org/10.1023/B:BIOP.0000022252.70836.fc

Munns R (2005) Genes and salt tolerance: bringing them together. New Phytol 167:645–663. https://doi.org/10.1111/j.1469-8137.2005.01487.x

Munns R, Schachtman D, Condon A (1995) The significance of a two-phase growth response to salinity in wheat and barley. Aust J Plant Physiol 22:561–569. https://doi.org/10.1071/PP9950561

Munns R, Rawson H (1999) Effect of salinity on salt accumulation and reproductive development in the apical meristem of wheat and barley. Funct Plant Biol 26:459–464. https://doi.org/10.1071/PP99049

Munns R, James R (2003) Screening methods for salinity tolerance: a case study with tetraploid wheat. Plant Soil 253:201–218. https://doi.org/10.1023/A:1024553303144

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681. https://doi.org/10.1146/annurev.arplant.59.032607.092911

Mushtaq M, Bhat JA, Mir ZA, Sakina A, Ali S, Singh AK, Tyagi A, Salgotra RK, Dar AA, Bhat R (2018) CRISPR/Cas approach: A new way of looking at plant-abiotic interactions. J Plant Physiol 224–225:156–162. https://doi.org/10.1016/j.jplph.2018.04.001

Nakajima Y, Yamamoto T, Muranaka T, Oeda K (1999) Genetic variation of petaloid male-sterile cytoplasm ofcarrots revealed by sequence-tagged sites (STSs). Theor Appl Genet 99:837–843

Nascimento WM, Vieira JV, Silva GO, Reitsma KR, Cantliffe DJ (2008) Carrot seed germination at high temperature: effect of genotype and association with ethylene production. HortScience 43:1538–1543

Neta-Sharir I, Isaacson T, Lurie S, Weiss D (2005) Dual role for tomato heat shock protein 21: protecting photosystem II from oxidative stress and promoting color changes during fruit maturation. Plant Cell 17:1829. https://doi.org/10.1105/tpc.105.031914

Nissinen AI, Lemmetty A, Pihlava J-M, Jauhiainen L, Munyaneza JE, Vanhala P (2012) Effects of carrot psyllid (*Trioza apicalis*) feeding on carrot yield and content of sugars and phenolic compounds. Ann Appl Biol 161:68–80

Noble C, Rogers M (1992) Arguments for the use of physiological criteria for improving the salt tolerance in crops. Plant Soil 146:99–107. https://doi.org/10.1007/BF00012001

Nogales A, Nobre T, Cardoso HG, Muñoz-Sanhueza L, Valadas V, Campos MD, Arnholdt-Schmitt B (2016) Allelic variation on *DcAOX1* gene in carrot (*Daucus carota* L.): An interesting simple sequence repeat in a highly variable intron. Plant Gene 5:49–55. https://doi.org/10.1016/j.plgene.2015.11.001

Noori SAS, Sokhansanj A (2008) Wheat plants containing an osmotin gene show enhanced ability to produce roots at high NaCl concentration. Russ J Plant Physiol 55:256–258. https://doi.org/10.1134/s1021443708020143

Osakabe Y, Osakabe K (2017) Genome editing to improve abiotic stress responses in plants. Prog in Mol Biol and Transl Sci, 99–109 doi:10.1016/bs.pmbts.2017.03.007

Pachauri R, Reisinger A (2007) Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, Switzerland

Park H, Ko E, Jang E, Park S, Lee J, Ahn Y-J (2013) Expression of *DcHsp17.7*, a small heat shock protein gene in carrot (*Daucus carota* L.) Hort Environ Biotechnol 54:121–127. https://doi.org/10.1016/j.nbt.2011.04.002

Parmar N, Singh KH, Sharma D, Singh L, Kumar P, Nanjundan J, Khan YJ, Chauhan DK, Thakur AK (2017) Genetic engineering strategies for biotic and abiotic stress tolerance and quality enhancement in horticultural crops: a comprehensive review. 3Biotechnol 7:239. https://doi.org/10.1007/s13205-017-0870-y

Palta J, Simon G (2004) Developing and successfully implementing a strategy for breeding frost-hardy carrots. HortScience 39:880

Parsons J, Matthews W, Iorizzo M et al (2015) *Meloidogyne incognita* nematode resistance QTL in carrot. Mol Breed 35:114

Peterson CE, Simon PW (1986) Carrot breeding. In: Vegetable Breeding (ed) Bassett MJ. Crops.AVI, Westport, CN, pp 321–356

Radić V, Beatović D, Mrđa J (2007) Salt tolerance of corn genotypes (*Zea mays* L.) during germination and later growth. J Agri Sci 52:115–120. https://doi.org/10.2298/JAS0702115R

Richards RA, Dennett CW, Qualset CO, Epstein E, Norlyn JD, Winslow MD (1987) Variation in yield of grain and biomass in wheat, barley, and triticale in a salt-affected field. Field Crops Res 15:277–287. https://doi.org/10.1016/0378-4290(87)90017-7

Rockström J, Falkenmark M (2000) Semiarid crop production from a hydrological perspective: gap between potential and actual yields. Crit Rev Plant Sci 19:319–346. https://doi.org/10.1080/07352680091139259

Rodriguez R, Redman R (2005) Balancing the generation and elimination of reactive oxygen species. Proc Natl Acad Sci USA 102:3175. https://doi.org/10.1073/pnas.0500367102

Ruhlman T, Lee S-B, Jansen RK, Hostetler JB, Tallon LJ, Town CD, Daniell H (2006) Complete plastid genome sequence of *Daucus carota*: Implications for biotechnology and phylogeny of angiosperms. BMC Genom 7:222

Sakamoto A, Murata N (2002) The role of glycine betaine in the protection of plants from stress: clues from transgenic plants. Plant, Cell Environ 25:163–171. https://doi.org/10.1046/j.0016-8025.2001.00790.x

Santos CAF, Simon PW (2002) QTL analyses reveal clustered loci for accumulation of major provitamin A carotenes and lycopene in carrot roots. Mol Genet Genom 268:122–129

Savicka M, Shkute N (2010) Effects of high temperature on malondialdehyde content, superoxide production and growth changes in wheat seedlings (*Triticum aestivum* L.). Ekologija 56: https://doi.org/10.2478/v10055-010-0004-x

Schmidhalter U, Oertli J (1991) Transpiration/biomass ratio for carrots as affected by salinity, nutrient supply and soil aeration. Plant Soil 135:125–132

Schöffl F, Prändl R, Reindl A (1999) Molecular responses to heat stress. In: Shinozaki K, Yamaguchi-Shinozaki K (eds) Molecular Responses to Cold, Drought, Heat and Salt Stress in Higher Plants. Landes Co, Austin, TX, pp 81–98

Schulz B, Westphal L, Wricke G (1994) Linkage groupsof isozymes, RFLP and RAPD markers in carrot(*Daucus carota* L. *sativus*). Euphytica 74:67–76

Shahid M, Balal R, Pervez M, Abbas T, Ashfaq M, Ghazanfar U, Afzal M, Rashid A, Garcia-Sanchez F, Mattson N (2012) Differential response of pea (*Pisum sativum* L.) genotypes to salt stress in relation to the growth, physiological attributes antioxidant activity and organic solutes. Aust J Crop Sci 6:828–838

Shannon M (1985) Principles and strategies in breeding for higher salt tolerance. Plant Soil 89:227–241. https://doi.org/10.1007/BF02182244

Shi J, Gao H, Wang H, Lafitte HR, Archibald RL, Yang M, Hakimi SM, Mo H, Habben JE (2017) ARGOS8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. Plant Biotechnol J 15:207–216. https://doi.org/10.1111/pbi.12603

Shi J, Habben JE, Archibald RL, Drummond BJ, Chamberlin MA, Williams RW, Lafitte HR, Weers BP (2015) Overexpression of ARGOS genes modifies plant sensitivity to ethylene, leading to improved drought tolerance in both arabidopsis and maize. Plant Physiol 169:266–282. https://doi.org/10.1104/pp.15.00780

Shinozaki K, Yamaguchi-Shinozaki K (2006) Gene networks involved in drought stress response and tolerance. J Exp Bot 58:221–227. https://doi.org/10.1093/jxb/erl164

Shiota H, Kamada H (2000) Acquisition of desiccation tolerance by cultured carrot cells upon ectopic expression of C-ABI3, a carrot homolog of ABI3. J Plant Physiol 156:510–515. https://doi.org/10.1016/s0176-1617(00)80166-2

Shomer-Ilan A, Jones G, Paleg L (1991) In vitro thermal and salt stability of pyruvate kinase are increased by proline analogues and trigonelline. Funct Plant Biol 18:279–286. https://doi.org/10.1071/PP9910279

Shu S, Gao P, Li L, Yuan Y, Sun J, Guo S (2016) Abscisic acid-induced H_2O_2 accumulation enhances antioxidant capacity in pumpkin-grafted cucumber leaves under $Ca(NO_3)_2$ stress. Front Plant Sci 7:1489. https://doi.org/10.3389/fpls.2016.01489

Sies H, Stahl W (1995) Vitamins E and C, beta-carotene, and other carotenoids as antioxidants. Am J Clin Nutr 62:1315S–1321S. https://doi.org/10.1093/ajcn/62.6.1315S

Simon PW (2000) Domestication, historical development, and modern breeding of carrot. Plant Breed Rev 19:157–190

Simon PW (2019) Economic and academic importance. In: Simon PW, Iorizzo M, Grzebelus D, Baranski R (eds) The Carrot Genome. Springer, Cham, Switzerland, pp 1–8

Simon PW, Goldman IL (2007) Carrot. In: Singh RJ (ed) Genetic Resources, chromosome Engineering, and Crop Improvement Series, vol 3. CRC Press. Boca Raton, FL, pp 497–517
Simon PW, Grzebelus D (2019) Carrot genetics and breeding. In: Geoffriau E, Simon PW (eds) Carrot and other cultivated Apiaceae, CABI, Oxford, UK, in press
Simon PW, Matthews WC, Roberts PA (2000) Evidence for simply inherited dominant resistance to *Meloidogyne javanica* in carrot. Theor Appl Genet 100:735–742
Simon PW, Freeman RE, Vieira JV, Boiteux LS, Briard M, Nothnagel T, Michalik B, Kwon YS (2008) Carrot. In: Prohens J, Nuez F (eds) Handbook of Plant Breeding: Vegetables II: Fabaceae, Liliaceae, Solanaceae, and Umbelliferae. Springer, New York, pp 327–357
Simon PW, Geoffriau E, Ellison S, Iorizzo M (2019) Carrot carotenoid genetics and genomics. In: Simon PW, Iorizzo M, Grzebelus D, Baranski R (eds) The Carrot Genome. Springer, Cham, Switzerland, pp 247–260
Simpson K, Fuentes P, Quiroz-Iturra LF, Flores-Ortiz C, Contreras R, Handford M, Stange C (2018) Unraveling the induction of phytoene synthase 2 expression by salt stress and abscisic acid in *Daucus carota*. J Exp Bot 69:4113–4126. https://doi.org/10.1093/jxb/ery207
Singh NK, Handa AK, Hasegawa PM, Bressan RA (1985) Proteins associated with adaptation of cultured tobacco cells to NaCl. Plant Physiol 79:126–137. https://doi.org/10.1104/pp.79.1.126
Singsaas EL, Lerdau M, Winter K, Sharkey TD (1997) Isoprene increases thermotolerance of isoprene-emitting species. Plant Physiol 115:1413. https://doi.org/10.1104/pp.115.4.1413
Sorensen JN, Jorgensen U, Kuhn BF (1997) Drought effects on the marketable and nutritional quality of carrots. J Sci Food Agri 74:379–391
Spooner DM (2019) *Daucus*: Taxonomy, phylogeny, distribution. In: Simon PW, Iorizzo M, Grzebelus D, Baranski R (eds) The Carrot Genome. Springer, Switzerland, pp 9–26
Sreenivasulu N, Sopory S, Kishor P (2007) Deciphering the regulatory mechanisms of abiotic stress tolerance in plants by genomic approaches. Gene 388:1–13. https://doi.org/10.1016/j.gene.2006.10.009
Stahl W, Sies H (2003) Antioxidant activity of carotenoids. Fat Soluble Vitam Old Mol Nov Prop 24:345–351. https://doi.org/10.1016/S0098-2997(03)00030-X
Stetler RA, Gan Y, Zhang W, Liou AK, Gao Y, Cao G, Chen J (2010) Heat shock proteins: cellular and molecular mechanisms in the central nervous system. Prog Neurobiol 92:184–211. https://doi.org/10.1016/j.pneurobio.2010.05.002
Subramanyam K, Sailaja KV, Subramanyam K, Muralidhara Rao D, Lakshmidevi K (2010) Ectopic expression of an osmotin gene leads to enhanced salt tolerance in transgenic chilli pepper (*Capsicum annum* L.). Plant Cell Tiss Org Cult105(2), 181–192. https://doi.org/10.1007/s11240-010-9850-1
Tan W, Meng Q wei, Brestic M, Olsovska K, Yang X (2011) Photosynthesis is improved by exogenous calcium in heat-stressed tobacco plants. J Plant Physiol 168:2063–2071. https://doi.org/10.1016/j.jplph.2011.06.009
Tester M, Davenport R (2003) Na^+ tolerance and Na^+ transport in higher plants. Ann Bot 91:503–527. https://doi.org/10.1093/aob/mcg058
Turner S, Ellison S, Senalik DA, Simon PW et al (2018) An automated, high-throughput image analysis pipeline enables genetic studies of shoot and root morphology in carrot (*Daucus carota* L.). Front Plant Sci 9:1703
Turner SD, Maurizio PL, Valdar W, Yandell BS, Simon PW (2017) Dissecting the genetic architecture of shoot growth in carrot (*Daucus carota* L.) using a diallel mating design. Genes Genom Genet 8:411–426
Vieira JV, Della Vecchia P, Ikuta H (1983) Cenoura 'Brasilia'. Hort Bras 1:42
Vivek BS, Simon PW (1999) Linkage relationshipsamong molecular markers and storage root traits ofcarrot (*Daucus carota L.* ssp. *sativus*). Theor ApplGenet 99:58–64
Wahid A, Gelani S, Ashraf M, Foolad MR (2007) Heat tolerance in plants: an overview. Environ Exp Bot 61:199–223. https://doi.org/10.1016/j.envexpbot.2007.05.011

Wang H, Ou C-G, Zhuang F-Y, Ma Z-G (2014) The dual role of phytoene synthase genes in carotenogenesis in carrot roots and leaves. Mol Breed 34:2065–2079. https://doi.org/10.1007/s11032-014-0163-7

Westphal L, Wricke G (1991) Genetic and linkage analysis of isozyme loci in *Daucus carota* L. Euphytica 56:259–267

Wohlfeiler J, Alessandro MS, Galmarini CR (2019) Multiallelic digenic control of vernalization requirement in carrot (*Daucus carota* L.). Euphytica 215:1–10

Xu Z-S, Tan HW, Wang F, Hou XL, Xiong AS (2014) CarrotDB: a genomic and transcriptomic database for carrot. Database (Oxford) 2014 doi:10.1093/database/bau096

Xu Z-S, Feng K, Xiong A-S (2019) CRISPR/Cas9-mediated multiply targeted mutagenesis in orange and purple carrot plants. Mol Biotechnol 61:191–199

Yau Y, Simon PW (2003) A 2.5-kb insert eliminates acid soluble invertase isozyme II transcript in carrot (*Daucus carota* L.) roots, causing high sucrose accumulation. Plant Mol Biol 53:151–162

Yau YY, Santos K, Simon PW (2005) Molecular tagging and selection for sugar type in carrot roots with codominant, PCR-based markers. Mol Breed 16:1–10

Yeo A, Flowers T (1986) Salinity resistance in rice (*Oryza sativa* L.) and a pyramiding approach to breeding varieties for saline soils. Aust J Plant Physiol 13:161–173. https://doi.org/10.1071/PP9860161

Yildiz M, Willis DK, Cavagnaro PF, Iorizzo M, Abak K, Simon PW (2013) Expression and mapping of anthocyaninbiosynthesis genes in carrot. Theor Appl Genet 126:1689–1702

Yoshida Y, Marubodee R, Ogiso-Tanaka E, Iseki K, Isemura T, Takahashi Y, Muto C, Naito K, Kaga A, Okuno K, Ehara H, Tomooka N (2016) Salt tolerance in wild relatives of adzuki bean, *Vigna angularis* (Willd.) Ohwi et Ohashi. Genet Resour Crop Evol 63:627–637. https://doi.org/10.1007/s10722-015-0272-0

Chapter 7
Allium Functional Genomic Development for Future Climatic Changes

Mostafa Abdelrahman

7.1 Introduction

Allium plants represent the most economically important and representative genus of the Alliaceae family. References to these plants in the Quran and Bible reflect their significance to ancient civilizations both as flavorful foods and healing herbs. *Allium* is a huge genus (850 species) that is spread widely across the Northern Hemisphere from the boreal zone to the dry subtropics. A region of high species diversity spreads from the Mediterranean Basin to Central Asia, and a second smaller center of species diversity is located in North America (Kamenetsky and Rabinowitch 2006; Fritsch et al. 2010; Abdelrahman et al. 2016, Abdelrahman et al. 2017d) (Fig. 7.1). The *Allium* species have adapted to diverse ecological niches, which led to the development of several distinct morphotypes, resulting in difficulties in classification and taxonomy of *Allium* (Gregory et al. 1998; Abdelrahman et al. 2015). A multidisciplinary approach, including morphological and anatomical examinations, and systematic studies using molecular and biochemical markers have led to an infrageneric classification of *Allium* species into six subgenera (*Melanocrommyum*, *Rhizirideum*, *Caloscordum*, *Bromatorrhiza*, *Amerallium*, and *Allium*) and 43 sections (Hanelt et al. 1992; Hanelt and Fritsch 1994; Khassanov 1996; Friesen et al. 1999; Fritsch and Friesen 2002; Ricroch et al. 2005; Fritsch et al. 2010). Many species of *Allium* genus have high economic importance, including vegetables [bulb onion (*A. cepa*), shallot (*A. cepa* L. Aggregatum group), Japanese bunching onion (*A. fistulosum*), garlic (*A. sativum*), leek, kurrat, and great-headed garlic (*A. ampeloprasum*), chives (*A. schoenoprasum*), Chinese chives (*A. tuberosum*)], and ornamentals [(*A. giganteum*, *A. aflatunense*, *A. karataviense*)]. Also, about two dozens of *Allium*

M. Abdelrahman (✉)
Arid Land Research Center, Tottori University, Tottori 680-0001, Japan
e-mail: meettoo2000@tottori-u.ac.jp; meettoo2000@ige.tohoku.ac.jp

Botany Department, Faculty of Sciences, Aswan University, Aswan 81528, Egypt

C. Kole (ed.), *Genomic Designing of Climate-Smart Vegetable Crops*,
https://doi.org/10.1007/978-3-319-97415-6_7

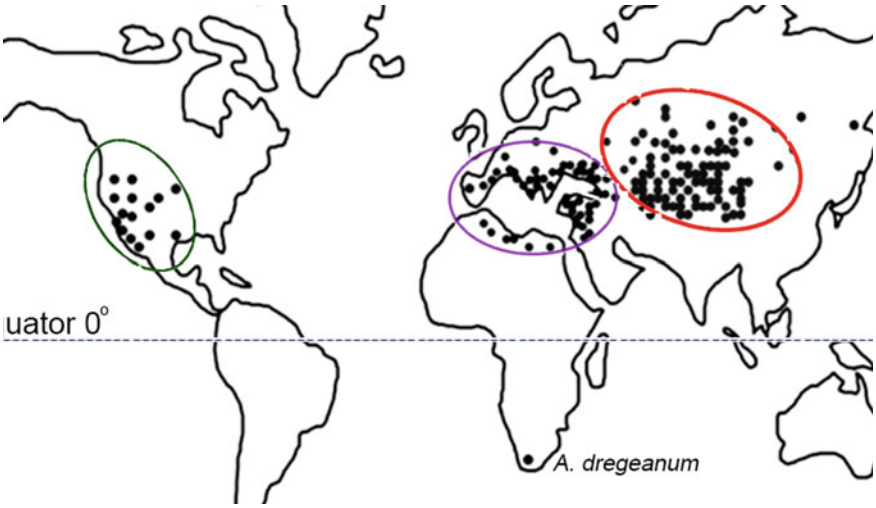

Fig. 7.1 Schematic diagram of the geographical distribution of *Allium* species based on literature. Color circles indicate the diversity centers extending from Central Asia to the Mediterranean Basin and Western North America

species are locally collected or cultivated as highly valued seasonings and medicinal plants. However, detailed information about the widespread use of these species remains incomplete (Khassanov 1996; Friesen et al. 2000; Fritsch and Friesen 2002; Keusgen et al. 2006).

During initial domestication, many immediate ancestors of *Allium* species have either been changed or lost. For instance, increasing numbers of different repetitive DNAs or retrotransposons, the lower GC content together with a minor DNA restructuring by point mutation is accountable for the enormousness size and complexity of the genome of many *Allium* crop species. For example, 2C DNA amounts per genome in 75 *Allium* species range between 16.93 and 63.57 pg (Ohri and Pistrick 2001), and onion has 16,415 megabase pairs (MbPs) of DNA per 1C nucleus which is 6 times higher than maize (*Zea mays*) and 16 times than rice (*Oryza sativa*), while garlic has ~15, 901 Mbp (Orhi et al. 1998). Also, the GC content of onion DNA is 32%, which is considered as the lowest among angiosperms (Kirk et al. 1970). Such a complicated genome reorganization is estimated to cause the speciation of *Allium*, which is the primary factor for reproductive isolation followed by the enlargement of habitat range. Genetic shifts and severe unbalanced selection pressure by breeders and farmers resulted in the loss of many useful agronomic traits for modern agriculture; therefore, genes of potentially useful characteristics were lost or are not readily available for crop improvement (Friesen et al. 2000; Fritsch and Friesen 2002; Kamenetsky and Rabinowitch 2006; Keusgen et al. 2006; Abdelrahman et al. 2014; Abdelrahman et al. 2018).

With the rise of next-generation sequencing (NGS) technologies, an increase in the speed and efficiency of DNA sequencing with higher throughputs and greater genome

coverage became achievable in many plant species including *Allium* (Abdelrahman et al. 2017a, b, c, d, f; Valliyodan et al. 2017; Yuan et al. 2017). These technologies led to the initial waves of crop genome sequences and facilitated the development of gene expression atlases and increased our understanding of the signaling pathways involved in the responses of plants to abiotic and biotic stressors (Rothberg et al. 2011; Pavlovich 2017; Abdelrahman et al. 2019b). The *Allium* international research community has developed several types of genetic stocks and applied these stocks to the latest modern technologies, which will be a milestone to accelerate *Allium* functional genomic studies. In this chapter, we will discuss the recent studies in *Allium* functional genomics as an innovative means of targeting the gene and bioactive metabolites responsible for the development of elite *Allium* varieties with unique chemical constituents and, subsequently, improved plant stress tolerance and human health benefits.

7.2 Organosulfur Compounds: A Prospective Active Ingredient for *Allium* Breeding

The *Allium* consumption as ethnomedicine or food ingredient is mostly associated with its nutritional and functional properties, which is mainly attributed to a variety of secondary metabolites (Caruso et al. 2014; Abdelrahman et al. 2016; Abdelrahman et al. 2019a). Among these secondary metabolites, organosulfur compounds are essential substances in terms of both biological activity and chemotaxonomic value of *Allium* species (Rose et al. 2005; Mostafa et al. 2013). There are four representatives of organosulfur compounds in *Allium* species, including (+)-S-(propyl)-L-cysteine sulfoxide (Propiin), (+)-S-(1-propenyl)-L-cysteine sulfoxide (Isoalliin), (+)-S-methyl-L-cysteine sulfoxide (Methiin), and (+)-S-(2-propenyl)-L-cysteine sulfoxide (Alliin) (Freeman and Whenham 1975; Hashimoto et al. 1984) (Fig. 7.2). These compounds are characteristics of each species and are generated by chemical transformation and cleavage of odorless, S-alk(en)yl cysteine sulphoxide precursors by the enzymes alliinase and lachrymatory-factor synthase (Jones et al. 2004). While S-alk(en)yl cysteine sulphoxides are found in the cytosol of the mesophyll tissue, alliinase is located in the vacuole of the vascular bundle sheath (Lancaster and Collin 1981). Once tissue being damaged by crashing or cutting alliinase is released and contact with S-alk(en)yl cysteine sulphoxides to cleave the C–S bond and generate sulfenic acid which is rapidly converted to thiosulfinates by non-enzymatic self-condensation (Yoshimoto and Saito 2017). Methiin is present in most of the *Allium* species and some Brassicaceae, alliin is characteristic of garlic, isoalliin is characteristic of onion and chive, while propiin is characteristic of onion, but it can be found in a minor content in most of the *Allium* species. Although methiin is present in less than 20% of total precursors in *A. cepa*, *A. sativum*, *A. ampeloprasum*, *A. proliferum*, *A. galanthum*, and *A. tuberosum*, however, some *Allium* species have a high content of methiin, which make them inappropriate for human consumption due to the strong

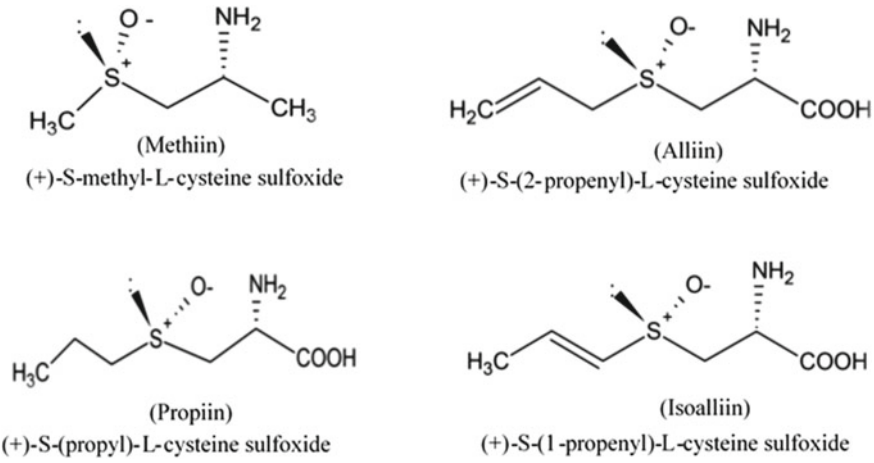

Fig. 7.2 Chemical structure of the four major cysteine sulfoxide compounds in *Allium* species

pungent smell (Kamenetsky and Rabinowitch 2006; Pratt 2010, Ramirez et al. 2017; Putnik et al. 2019).

Furthermore, chemotaxonomy of 40 *Allium* species from different subgenera revealed at least seven different chemotypes and showed specific arrays of volatile organosulfur compounds in the rhizomatous species (Storsberg et al. 2003; Kamenetsky and Rabinowitch 2006). This classification can contribute to a better selection of wild species for breeding experiments to improve taste, aroma, and medicinal properties of interspecific *Allium* hybrids. For instance, a recent study using chromosomal addition lines revealed an increase in organosulfur compounds, including gamma-glutamyl-PrenCS, S-2-carboxypropyl glutathione (2-CPGTH) and methiin, cycloalliin in *A. fistulosum* with extra chromosome 2A from shallot (Abdelrahman et al. 2019a).

Differences between cultivar and species in flavor characteristics mostly arose from variability in their sulfur uptake and metabolism, and the availability of sulfur is one of the factors that control the biosynthesis of each flavor precursor (Fritsch 2001; Storsberg et al. 2003; Kamenetsky and Rabinowitch 2006). The *Allium* plants synthesize organic sulfur compounds using the inorganic sulfate absorbed from the soil. Sulfate is converted into sulfite by adenosine 5′-phosphosulfate reductase (EC 1.8.4.9), and the latter converted into sulfide by sulfite reductase enzyme (EC 1.8.7.1). Sulfide is incorporated into cysteine, which subsequently undergoes two conversions, glutamylation and glycosylation, to yield glutathione (Turnbull et al. 1980; Kodera et al. 2017). Although the biosynthetic pathway of the flavor precursors, including (+)-S-alk(en)yl cysteine sulfoxides and their γ-glutamyl peptide relatives, has been published (Lancaster and Shaw 1989), there is still ambiguity about several stages, and whether the same pathway is followed in all tissues. It has been proposed that the biosynthesis of the flavor precursors in *Alliums* started with S-alk(en)ylation of the cysteine in glutathione, followed by transpeptidation to remove the glycyl group,

oxidation to the cysteine sulfoxides, and, finally, removal of the glutamyl group to yield cysteine sulfoxides (Lancaster and Shaw 1989; Lancaster et al. 1989; Block 1992; Prince et al. 1997). An alternative biosynthetic pathway ignores glutathione in favor of straight thioalk(en)ylation of O-acetyl serine or alk(en)ylation of cysteine, followed by oxidation to a sulphoxide. In both of the pathways, few of the proposed enzymes involved in the biosynthesis of S-alk(en)yl-L-cysteine sulfoxides from *Alliums* have not been identified. A recent subcellular localizations and kinetic properties of three γ-Glutamyl transpeptidases (GGT; EC 2.3.2.2) genes including *AsGGT1*, *AsGGT2*, and *AsGGT3* isolated from garlic suggested that these genes may contribute differently to the biosynthesis of alliin in garlic (Yoshimoto et al. 2015). To date, several studies have been conducted to characterize and identify GGTs in *Allium* plants, for instance, AcGGT partially purified from onion showed high substrate specificity to γ-glutamyl compounds, a putative intermediates of S-alk(en)yl-L-cysteine sulfoxide biosynthesis, suggesting the involvement of AcGGT in the biosynthesis of S-alk(en)yl-L-cysteine sulfoxides in onion (Lancaster and Shaw 1994). A partial cDNA of *AsGGT*, which has high sequence homology to *AcGGT*, was isolated from garlic, and its expression profiles suggested that AsGGT may play a role in synthesizing S-alk(en)yl-L-cysteine sulfoxides in garlic cloves during cold storage (Cho et al. 2012). Future investigations of the in vivo functions of different GGT will offer a better understanding of the molecular mechanisms underlying the biosynthesis of alliin and other cysteine sulfoxide compounds in *Allium*, which can be applied to future metabolic engineering of crop plants.

The role of organosulfur compounds in *Allium* abiotic stress tolerance is still unclear; however, some few evidences reported that *Allium* species and landraces grown under stress conditions exhibited high level of organosulfur compounds. For example, a comparative targeted metabolite profiling and transcriptome landscapes of tropical shallot doubled haploid (stress-tolerant) and cultivated onion doubled haploid, and their F_1 hybrid revealed several key genes and metabolites related to organosulfur were introgressed in abiotic stress response were upregulated shallot and F_1 genotypes as compared onion (Abdelrahman et al. 2015). Also the additional chromosome from shallot to Japanese bunching onion induced organosulfur compound accumulation under summer conditions (Masamura et al. 2011). Similarly, shallot landraces derived from Indonesia possessed high levels of methiin and isoalliin in comparison with different onion varieties (Ariyanti et al. 2018). In addition, a comparative study of antioxidant activities and organosulfur compounds in garlic, elephant garlic, and onion demonstrated a significant positive correlation between organosulfur compounds and antioxidant capacity in *Allium* crops (Kim et al. 2018). Nevertheless, there are no direct studies that addressed the in-depth role of organosulfur compounds in *Allium* abiotic stress tolerance, which remain to be a future task.

7.3 Steroidal Saponins in *Allium* Species: Anticancer, Antimicrobial, and Biosynthesis Pathway

Although organosulfur compounds have been considered a key component of *Allium* plants' medicinal properties, various researchers tend to attribute the prospective medicinal benefits of *Allium* plants to other constituents, such as polyphenolic compounds, especially flavonoids, steroidal saponins as well as sugars (Lanzotti 2005; Stajner et al. 2006; Lanzotti et al. 2012; Abdelrahman et al. 2017e). The genus *Allium* is a rich source of steroidal saponins, which can be classified into spirostanol, furostanol, and cholestane saponins based on their sapogenin structure (Challinor and De Voss 2013; Mostafa et al. 2013; Abdelrahman et al. 2017d). Apart from the Amaryllidaceae family, steroidal saponins are also broadly spread in other monocot families, such as Costaceae, Asparagaceae, Liliaceae, Dioscoreaceae, Melanthiaceae, and Smilacaceae. These saponin compounds have also been reported in some dicotyledonous angiosperms: Zygophyllaceae, Plantaginaceae, Solanaceae, and Fabaceae. The earliest reports on *Allium* saponins date back to the 1970s through the identification of alliogenin in the bulbs of *A. giganteum* (Khristulas et al. 1970) and diosgenin in *A. albidum* (Kereselidze et al. 1970), which was followed by first chemical survey of saponins from the *Allium* genus by Kravets in (Kravets et al. 1990), and Lanzotti in (Lanzotti 2005), (Kravets et al. 1990, Lanzotti 2005). Since then, a huge number of new saponin compounds have been revealed. The *Allium* saponins are mainly bi- or mono-desmosides; however, a tri-desmodic cholestane glycoside has been described in the bulbs of *A. macleanii* (Inoue et al. 1995). The sugar chain in *Allium* saponins consists of branched or linear chains made up most often of glucose, galactose, rhamnose, arabinose, and xylose units (Mostafa et al. 2013; Sobolewska et al. 2016).

Saponins are considered accountable for various pharmacological properties of several plants, and they are recognized as bioactive constituents of *Allium* species (Sobolewska et al. 2016). There have been several reports addressing the pharmacological activities of steroidal saponins, including cytotoxic, antithrombotic, antifungal, anti-inflammatory, and immunomodulatory effects (Sparg et al. 2004; Sun et al. 2009; Lanzotti et al. 2012; Abdelrahman et al. 2017e). Saponins are potential anticancer molecules, and the induction of apoptosis by saponins has been defined in several studies, including inhibition of cancer migration (Sun et al. 2010; Zhao et al. 2014) and proliferation (Beit-Yannai et al. 2011; Zhang et al. 2012). Steroidal saponins isolated from different *Allium* species displayed amazing cytotoxic activities against different animal and human cancer cell lines, such as 4T1 breast carcinoma, B16 melanoma, hepatocellular carcinoma HepG2, fibroblast 3T3-L1, and pheochromocytoma PC12 cell lines (Chen et al. 2009; Luo et al. 2011; Yu et al. 2015). In vitro examination of the cytotoxic activity of Cepa2 steroidal saponin, isolated from the dry roots of shallot against P3U1 myeloma cancer cell line showed its high efficiency as an anticancer with 91.13% reduction in P3U1 cell viability (Abdelrahman et al. 2017e). The reduction of cell viability was correlated with the increase in reactive oxygen species levels in Cepa2-treated P3U1 cells (Abdelrhaman

et al. Abdelrahman et al. 2017e). Similarly, Tuberoside M isolated from the seeds of *A. tuberosum* and F-gitonin isolated from the fresh bulbs of *A. jesdianum* inhibited the cancer cells growth, with $IC_{50} = 6.8$ and 1.5 μg/mL, respectively (Sang et al. 2001; Mimaki et al. Mimaki et al. 1999). More recently, the cytotoxic substance of *A. chinense* saponins (ACSs) inhibited the proliferation, cell migration, and colony formation of 4T1 and B16 cells in a dose-dependent manner (Yu et al. 2015). These studies above provide clear evidence for the anticancer activities of the natural saponin compounds isolated from *Allium* plants, and a strong basis for in-depth investigations for the development of novel anticancer drugs.

With increasing concern about the negative impacts of climate change on the development of plant disease epidemics and altering the interactions between plant and pathogens, greater effort toward improving plant disease resistance became a mandate. Although many steroidal saponin compounds isolated from diverse plant species have been reported to have antifungal activity, unfortunately, only a few studies have been performed so far on *Allium* steroidal glycosides antifungal properties (Mostafa et al. 2013; Sobolewska et al. 2016). Antifungal activity of *Allium* saponins is controlled by both the number and structure of the sugar residue and sapogenin type. Generally, saponins with spirostanol skeleton exhibited higher antifungal activity than furostanols (Mostafa et al. 2013). Lanzotti et al. (2012) provided a strong evidence for the significant differences in the potency of saponin compounds belonging to spirostane relative to furostane groups. For instance, gitogenin 3-*O*-tetrasaccharide and gigenin 3-*O*-trisaccharide, isolated from the bulbs of *A. sativum* var. Voghiera, were more active against *Trichoderma harzianum* and *Botrytis cinerea* than furostanol voghierosides isolated from the same plant (Lanzotti et al. 2012). Also, the sprirostanol Aginoside isolated from *A. nigrum* at 400 ppm completely inhibited the growth of *Botrytis squamosa* and *C. gloeosporioides*, and partially inhibited *F. oxysporum* f. sp. *cepae* and *F. oxysporum* f. sp. *radicis-lycopersici* (Mostafa et al. 2013). The influence of the structure of the sugar chain on the observed antifungal activity of Alliospirosides A isolated from the roots of shallot, inhibited a wide range of plant pathogenic fungi, including *Alternaria* ssp., *Botrytis* ssp., *Colletotrichum* spp., *Curvularia lunata*, *Epicoccum nigrum*, and *Fusarium* ssp. (Teshima et al. 2013). However, Alliospirosides A activity against *Fusarium* pathogens was relatively low in comparison with other phytopathogens (Teshima et al. 2013). Despite a large number of saponin compounds being isolated from different *Allium* species, little efforts have been invested in their antifungal activity. One of the main reasons for such drawback is the limited amount of the isolated pure compounds which are mostly being consumed through the identification and chemical structure elucidation methods by mass spectrometry and nuclear magnetic resonance (NMR).

In plants, steroidal saponins are mostly synthesized from lanosterol and cycloartenol via cholesterol and sitosterol, respectively. However, the steroidal saponin biosynthesis pathway in *Allium* has not been reported yet. Differential expression analysis of *Asparagus racemosus* fruit, leaves, and roots showed that expression of the transcripts involved in steroidal saponin biosynthesis is mainly upregulated in the leaf and root tissues, whereas triterpene saponins was dominated

in fruit and leaf tissues (Srivastava et al. 2018). In a recent study, Abdelrahman et al. (2017d) were able to isolate and identify Alliospiroside A saponin compound in *A. fistulosum* (FF) with additional chromosome 2A (FF2A) from shallot (AA) with potent role in defense mechanism against *Fusarium* pathogens. In addition, differential gene expression analyses of AA and FF2A as compared to FF (as a control) revealed a strong upregulation of the saponin downstream pathway, including glycosyltransferase, cytochrome P450, and beta-glucosidase in chromosome 2A (Abdelrahman et al. 2017d). An understanding of the biosynthesis-related genes and saponin compounds would facilitate the development of plants with unique saponin content and, subsequently, improved disease resistance.

7.4 Metabolomic and Transcriptomic Landscapes of *Allium* Crops Under Environmental Stress

The heavy yield losses in primary crops due to global warming and the increasing demand for food mean that there is a crucial need to improve food security (Abiala et al. 2018; Zhang et al. 2019). However, the development of abiotic stress-resilient crops requires an in-depth information about the biological processes that enable plants to survive in stressful environments, and this information can be achieved from "omic" studies, such as metabolomics, proteomics, transcriptomics, and genomics (Hirata et al. 2016; Abdelrahman et al. 2017b; Abdelrahman et al. 2018a, b; Galsurker et al. 2018; Wang et al. 2018). Unfortunately, there are limited studies addressing the *Allium* metabolome and transcriptome profiling in response to environmental stress, and thus the *Allium* international community needs further efforts in this regard. Transcriptome analysis between inner and outer scales of commercial brown onion cv. "Orlando" in response to the heat stress demonstrated that oxidation and lipid metabolism pathways, as well as cell-wall modification were highly expressed in the onion outer scale under heat stress (Galsurker et al. 2018). However, defense response-related genes such as those encoding antioxidative stress defense, heat shock proteins, or production of osmo-protectant metabolites were highly induced in the inner scale (Galsurker et al. 2018). These transcriptomic data led to a conceptual model that suggests consecutive processes for the development of desiccation and browning of the outer scale versus processes associated with defense response and heat tolerance in the inner scales (Galsurker et al. 2018). Transcriptome-based sequencing of cold-tolerant and cold-susceptible genotypes of onion under freezing and cold conditions indicated that several genes were significantly induced by freezing and cold stress in tolerant lines relative to susceptible genotype (Han et al. 2016). Among these transcript, genes encoding hypothetical proteins, zinc finger (ZIP) proteins, heat shock proteins (HSPs), and CBL-interacting protein kinase (CIPK), in addition to subset of transcription factors, particularly those that function as activators including dehydration-responsive element (DRE)-binding (DREB),

CBL, MYB, bZIP, zinc finger of *Arabidopsis thaliana* (ZAT), HSPs and basic helix-loop-helix (bHLH) were drastically changed during freezing and cold conditions (Han et al. 2016). Similarly, genome-wide transcriptome profiling analysis of garlic under low temperature stress indicated that enzyme-encoding genes, which significantly enriched the pathway "proteasome," are potentially involved in the garlic discoloration under low temperature stress, such as γ-glutamyltranspeptidase-, δ-aminolevulinic acid dehydratase-, and alliinase-encoding genes (Li et al. 2018). These stress-responsive genes are possibly responsible for the low-temperature-induced garlic discoloration (Li et al. 2018). Effects of salinity stress on the growth parameters and K^+/Na^+ ratio of *Allium* vegetables (Welsh onion and Wakegi) using diverse concentrations of seawater demonstrate stunting of plants; however, the rate of growth reduction under salinity stress varies widely among different *Allium* plants (Arakaki et al. 2014). The chlorophyll content as in term of SPAD values of the leaves of the Welsh onion decreased, whereas the SPAD value of the two types of Wakegi cultivars increased (Arakaki et al. 2014). In addition, the total sugar and phenolic contents increased significantly compared with the respective controls under seawater treatment (Arakaki et al. 2014). Environmental stress affects plant growth, thus identification of stress biomarkers is a major prerequisite for the breeding of stress-tolerant crops. In this regard, because of its high adaptability to subtropical and tropical environment, shallots are recognized as an important genetic resource for the breeding of common onion (Abdelrahman et al. 2015). Using liquid chromatography quadruple-mass spectrometer (LC-QqQ-MS), the bulb onion double haploid, shallot double haploid, and its F_1 hybrid were evaluated. In total, 113 targeted metabolites were detected, and the principal component analysis and volcano plot analysis clearly showed genotype-specific metabolites, which can be used as metabolic markers of environmental tolerance (Abdelrahman et al. 2015). Similarly, integrated transcriptome and metabolome analysis of *A. fistulosum* with additional chromosome 5A from shallot revealed an accumulation of several flavonoid compounds which are majorly involved in abiotic and biotic stress tolerances (Abdelrahman et al. 2019a). Also the increase in flavonoid pool in *A. fistulosum* with additional chromosome 5A from shallot was consistent with the upregulation of many upstream and downstream flavonoid biosynthesis and regulatory genes (Abdelrahman et al. 2019a). The above results confirmed that shallot can be a potential genetic resource for the improvement of onion stress tolerance. Likewise, Zhang et al. (2018) used transcriptome analysis of two contrasting dark-red and white onion cultivars, revealing that both flavonoid 3′,5′-hydroxylase (*F3′,5′H*) and dihydroflavonol 4-reductase (*DFR*) genes play major role in the biosynthesis of dark-red bulbs, and the expression levels of flavonol synthase (*FLS*) and *DFR* genes may act to block blue pigmentation. In addition, the positive variation in the F3′,5′H/F3′H ratio also affects onion bulb color diversity (Zhang et al. 2018). A recent study using comparative transcriptome analysis of cold-tolerant and sensitive bulb onions provides further information regarding the transcriptional changes underlying cold and freezing tolerance mechanisms in addition to molecular markers that would facilitate gene mapping and genetic diversity analysis (Han et al. 2016).

7.5 Future Aspects for *Allium* Functional Genomics

Allium transcriptomics and metabolomics will elucidate characteristic metabolites and their biosynthesis or regulatory related genes within different accessions, landraces, and cultivars. The individual bio-resource-specific metabolic patterns can be used for molecular breeding of *Allium* crops while the broad metabolic profiles of *Allium* bioresources can be used for integrated omics approaches. Further integrated omics approaches, e.g., correlation analysis between transcriptome and metabolome, linkage mapping, can elucidate the gene-to-metabolite networks in environmental responses or stress. In this regard, *Allium* transcriptome database (*Allium* TDB; http://alliumtdb.kazusa.or.jp/) provides a comprehensive information of the transcriptome analysis in different *Allium* species that can be used for further genetic and molecular breeding studies. The integration of metabolomics and transcriptomics will provide insight into the molecular mechanism of *Allium* metabolite biosynthesis, which can be used for elucidation of the molecular architecture underlying environmental responses and stress tolerance in *Allium*.

References

Abdelrahman M, Abdel-Motaal F, El-Sayed M, Jogaiah S, Shigyo M, Ito S, Tran LS (2016) Dissection of *Trichoderma longibrachiatum* induced-defense in onion (*Allium cepa* L.) against *Fusariumoxysporum* f. sp. *cepa* by target metabolite profiling. Plant Sci 246:128–138

Abdelrahman M, Burritt DJ, Gupta A, Tsujimoto H, Tran LSP (2019b) Heat stress effect on source-sink relationships and metabolome dynamics in wheat. J Exp Bot erz296

Abdelrahman M, Burritt DJ, Tran LP (2017a) The use of metabolomic quantitative trait locus mapping and osmotic adjustment traits for the improvement of crop yields under environmental stresses. Semin Cell Dev Biol 83:86–94

Abdelrahman M, El-Sayed M, Jogaiah S, Burritt DJ, Tran LP (2017b) The "STAY-GREEN" trait and phytohormone signaling networks in plants under heat stress. Plant Cell Rep 36:1009–1025

Abdelrahman M, El-Sayed M, Sato S, Hirakawa H, Ito SI, Tanaka K, Mine Y, Sugiyama N, Suzuki M, Yamauchi N, Shigyo M (2017d) RNA-sequencing-based transcriptome and biochemical analyses of steroidal saponin pathway in a complete set of *Allium fistulosum-A. cepa* monosomic addition lines. PLoS One 12:0181784

Abdelrahman M, Jogaiah S, Burritt DJ, Tran LP (2018a) Legume genetic resources and transcriptome dynamics under abiotic stress conditions. Plant, Cell Environ 41:1972–1983

Abdelrahman M, El-Sayed MA, Hashem A, AbdAllah EF, Alqarawi AA, Burritt DJ, Tran LP (2018b) Metabolomics and transcriptomics in legumes under phosphate deficiency in relation to nitrogen fixation by root nodules. Front Plant Sci 9:922

Abdelrahman M, Hirata S, Sawada Y, Hirai MY, Sato S, Hirakawa H, Mine Y, Tanaka K, Shigyo M (2019a)Widely targeted metabolome and transcriptome landscapes of *Allium fistulosum–A. cepa* chromosome addition lines revealed a flavonoid hot spot on chromosome 5A. Sci Rep 9:3541

Abdelrahman M, Hirata S, Ito SI, Yamauchi N, Shigyo M (2014) Compartmentation and localization of bioactive metabolites in different organs of *Allium roylei*. Biosci Biotechnol Biochem 78:1112–22

Abdelrahman M, Mahmoud HYAH, El-Sayed M, Tanaka S, Tran LS (2017e) Isolation and characterization of Cepa2, a natural alliospiroside A, from shallot (*Allium cepa* L. Aggregatum group) with anticancer activity. Plant Physiol Biochem 116:167–173

Abdelrahman M, Mitoma M, Ikeuchi T, Mori M, Murakami K, Ozaki Y, Matsumoto M, Uragami A, Kanno A (2017c) Differential gene expression analysis and SNP/InDel marker discovery in resistant wild *Asparagus kiusianus* and susceptible *A. officinalis* in response to *Phomopsis asparagi* infection. Data Brief 21:2117–2121

Abdelrahman M, Sawada Y, Nakabayashi R, Sato S, Hirakawa H, El-Sayed M, Hirai MY, Saito K, Yamauchi N, Shigyo M (2015) Integrating transcriptome and target metabolome variability in doubled haploids of *Allium cepa* for abiotic stress protection. Mol Breed 35:195

Abdelrahman M, Suzumura N, Mitoma M, Matsuo S, Ikeuchi T, Mori M, et al. (2017c) Comparative de novo transcriptome profiles in *Asparagus officinalis* and *A. kiusianus* during the early stage of *Phomopsis asparagi* infection. Sci Rep 7:2608

Abiala MA, Abdelrahman M, Burritt DJ, Tran LP (2018) Salt stress tolerance mechanisms and potential applications of legumes for sustainable reclamation of salt-degraded soils. Land Deg Dev 29:3812–3822

Ariyanti NA, Torikai K, Kirana RP, Hirata S, Sulistyaningsih E, Ito SI, Yamauchi N, Kobayashi N, Shigyo M (2018) Comparative study on phytochemical variations in Japanese F_1 varieties of bulb onions and South-East Asian shallot landraces. J Jpn Soc Hort Sci 87:63–72

Arakaki M, Takahashi M, Hossain MDA, Wada K (2014) Changes in sugar content and antioxidant activity of *Allium* vegetables by salinity-stress. Food Sci Technol Res 20:705–710

Beit-Yannai E, Ben-Shabat S, Goldschmidt N (2011) Antiproliferative activity of steroidal saponins from *Balanites aegyptiaca*: an in vitro study. Phytochem Lett 4:43–47

Block E (1992) The organosulfur chemistry of the genus *Allium* implications for the organic chemistry of sulfur. Angew Chem 31:1135–1178

Caruso G, Conti S, Villari G, Borrelli C, Melchionna G, Mintutolo M, Russo G, Amalfitano C (2014) Effects of transplanting time and plant density on yield, quality and antioxidant content of onion (*Allium cepa* L.) in southern Italy. Sci Hort 166:111–120

Challinor VL, De Voss JJ (2013) Open-chain steroidal glycosides, a diverse class of plant saponins. Nat Prod Rep 30:429–454

Chen HF, Wang GH, Luo Q (2009) Two new steroidal saponins from *Allium macrostemon* bunge and their cytotoxicity on different cancer cell lines. Molecules 14:2246–2253

Cho J, Park M, Choi D, Lee SK (2012) Cloning and expression of γ-glutamyl transpeptidase and its relationship to greening in crushed garlic (*Allium sativum*) cloves. J Sci Food Agri 92:253–257

Fattorusso E, Lanzotti V, Taglialatela-Scafati O, Di Rosa M, Ianaro A (2000) Cytotoxic saponins from bulbs of *Allium porrum* L. J Agri Food Chem 48:3455–3462

Freeman GG, Whenham RJ (1975) A survey of volatile components of some *Allium* species in terms of S-alk(en)yl-L-cysteine sulphoxides presents as flavor precursors. J Sci Food Agri 26:186–1886

Friesen N, Fritsch RM, Pollner S, Blattner FR (2000) Molecular and morphological evidence for an origin of the aberrant genus Milula within Himalayan species of *Allium* (Alliaceae). Mol Phylogenet Evol 17:209–218

Friesen N, Pollner S, Bachmann K, Blattner FR (1999) RAPDs and non-coding chloroplast DNA reveal a single origin of the cultivated *Allium fistulosum* from *A. altaicum* (Alliaceae). Amer J Bot 86:554–562

Fritsch R (2001) Taxonomy of the genus *Allium*: Contribution from IPK Gatersleben. Herbertia 56:19–50

Fritsch RM, Blattner FR, Gurushidze M (2010) New classification of *Allium* L. subg. Melanocrommyum (Webb & Berthel) Rouy (Alliaceae) based on molecular and morphological characters. Phyton 49:145–220

Fritsch RM, Friesen N (2002) Evolution, domestication and taxonomy. In: Rabinovitch HD, Currah L (eds) *Allium* crop science: recent advances. CAB international, Wallingford, UK, pp 5–27

Galsurker O, Doron-Faigenboim A, Teper-Bamnolker P, Daus A, Lers A, Eshel D (2018) Differential response to heat stress in outer and inner onion bulb scales. J Exp Bot 69:4047–4064

Gregory M, Fritsch RM, Friesen NW, Khassanov FO, McNeal DW (1998) Nomenclator alliorum. Allium Names and Synonyms a World Guide, Royal Botanic Gardens, Kew

Han J, Thamilarasan SK, Natarajan S, Park J-I, Chung M-Y, Nou I-S (2016) De Novo assembly and transcriptome analysis of bulb onion (*Allium cepa* L.) during cold acclimation using contrasting genotypes. PLoS One 11(9):0161987

Hanelt P, Fritsch RM (1994) Notes on infrageneric taxa in *Allium* L. Kew Bull 49:559–564

Hanelt P, Schultze-Motel J, Fritsch RM, Kruse J, Maass HI, Ohle H, Pistrick K (1992) Infrageneric grouping of *Allium*. The Gatersleben approach. In: Hanelt P, Hammer K, Knupffer H (eds) The genus *Allium*-taxonomic problems and genetic resources. Proceedings of an international symposium held at Gatersleben, Germany, pp 11–13. Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany, pp 107–123

Hashimoto S, Miyazawa M, Kameoka H (1984) Volatile flavor components of *Allium grayi* Regel. J Sci Food Agri 35:353–356

Hirata S, Abdelrahman M, Yamauchi N, Shigyo M (2016) Characteristics of chemical components in genetic resources of garlic *Allium sativum* collected from all over the world. Genet Res Crop Evol 63:35–45

Inoue T, Mimaki Y, Sashida Y, Nishino A, Satomi Y, Nishino H (1995) Steroidal glycosides from *Allium macleanii* and *A. senescens*, and their inhibitory activity on tumor promoter-induced phospholipid metabolism of HeLa cells. Phytochemistry 40:521–525

Jones MG, Hughes J, Tregova A, Milne J, Tomsett AB, Collin HA (2004) Biosynthesis of the flavour precursors of onion and garlic. J Exp Bot 55:1903–1918

Kamenetsky R, Rabinowitch HD (2006). The genus *Allium*: a developmental and horticultural analysis. Hort Rev 32:329–378

Kereselidze EV, Pkheidze TA, Kemertelidze EP (1970) Diosgenin from *Allium albidum*. Chem Nat Comp 6:378

Keusgen M, Fritsch RM, Hisoriev H, Kurbonova PA, Khassanov FO (2006) Wild *Allium* species (Alliaceae) used in folk medicine of Tajikistan and Uzbekistan. J Ethnobiol Ethnomed 2:18

Khassanov FO (1996) Conspectus of the wild growing *Allium* species of Middle Asia. In: Öztürk M, Seçmen Ö, Görk G (eds) Plant Life in Southwest and Central Asia. EGE University Press, Izmir, pp 141–159

Khristulas FS, Gorovits MB, Luchanskaya VN, Abubakirov NK (1970) A new steroid sapogenin from *Allium giganteum*. Chem Nat Comp 6:489

Kim S, Kim DB, Jin W, Park J, Yoon W, Lee Y, Kim S, Lee S, Kim S, Lee OH, Shin D, Yoo M (2018) Comparative studies of bioactive organosulphur compounds and antioxidant activities in garlic (*Allium sativum* L.), elephant garlic (*Allium ampeloprasum* L.) and onion (*Allium cepa* L.). Nat Prod Res 32:1193–1197

Kirk JTO, Rees H, Evans G (1970) Base composition of nuclear DNA with the genus *Allium*. Heredity 25:507–512

Kodera Y, Ushijima M, Amano H, Suzuki JI, Matsutomo T (2017) Chemical and biological properties of S-1-Propenyl-l-Cysteine in aged garlic extract. Molecules 22:570

Kravets SD, Vollerner YS, Gorovits MB, Abubakirov NK (1990) Steroids of the spirostan and furostan series from plants of the genus *Allium*. Chem Nat Comp 26:359–373

Lancaster JE, Collin HA (1981) Presence of alliinase in isolated vacuoles and of alkyl cysteine sulphoxides in the cytoplasm of bulbs of onion (*Allium cepa*). Plant Sci Lett 22:169–176

Lancaster JE, Reynolds PHS, Shaw ML, Dommisse EM, Munro J (1989) Intracellular localization of the biosynthetic pathway to flavour precursors in onion. Phytochemistry 28:461–464

Lancaster JE, Shaw ML (1989) γ-glutamyl peptides in the biosynthesis of S-alk(en)yl-L-cysteine sulphoxides (flavour precursors) in *Allium*. Phytochemistry 28:455–460

Lancaster JE, Shaw ML (1994) Characterization of purified γ-glutamyl transpeptidase in onions: evidence for in vivo role as a peptidase. Phytochemistry 36:1351–1358

Lanzotti V (2005) Bioactive saponins from *Allium* and Aster plants. Phytochem Rev 4:95–110

Lanzotti V, Barile E, Antignani V, Bonanomi G, Scala F (2012) Antifungal saponins from bulbs of garlic, *Allium sativum* L. var. Voghiera. Phytochemistry 78:126–134

Li N, Qiu Z, Lu X, Shi B, Sun X, Tang X, Qiao X (2018) Comparative transcriptome analysis of temperature-induced green discoloration in garlic. Intl J Genom 2018:6725728

Luo H, Huang J, Liao WG (2011) The antioxidant effects of garlic saponins protect PC12 cells from hypoxia-induced damage. Brit J Nutr 105:1164–1172

Masamura N, Yaguchi S, Ono Y, Nakajima T, Masuzaki S, Imai S, Yamauchi N, Shigyo M (2011) Characterization of amino acid and S-alk(en)yl-L-cysteine sulfoxide production in Japanese bunching onion carrying an extra chromosome of shallot. J Jpn Soc Hort Sci 80:322–333

Mimaki Y, Kuroda M, Sashida Y (1999) Steroidal saponins from the bulbs of *Allium aflatunense*. Nat Med 53:88–93

Mostafa A, Sudisha J, El-Sayed M, Ito SI, Ikeda T, Yamauchi N, Shigyo M (2013) Aginsodie saponin, a potent antifungal compound, and secondary metabolite analyses from *Allium nigrum* L. Phytochem Lett 6:274–280

Ohri D, Pistrick K (2001) Phenology and genome size variation in *Allium* L.-a tight correlation? Plant Biol 3:654–660

Orhi D, Fritsch RM, Hanelt P (1998) Evolution of genome size in *Allium* (Alliaceae). Plant Syst Evol 210:57–86

Pavlovich (2017) Computing in biotechnology: Omics and beyond. Computation and Modeling 35:479–480

Pratt DA (2010) Garlic and other *Alliums*. The lore and the science. By Eric Block. Angewandte Chemie International Edition, Wiley. Royal Society of Chemistry, Cambridge, 454, pp 7162–7162

Prince CL, Shuler ML, Yamada Y (1997) Altering flavor profiles in onion (*Allium cepa* L.) root cultures through directed biosynthesis. Biotechnol Progr 13:506–510

Putnik P, Gabrić D, Roohinejad S, Barba FJ, Granato D, Mallikarjunan K, Lorenzo MJ, Kovačević DB (2019) An overview of organosulfur compounds from *Allium* spp.: from processing and preservation to evaluation of their bioavailability, antimicrobial, and anti-inflammatory properties. Food Chem 276:680–691

Ramirez DA, Locatelli DA, González RE, Cavagnaro PF, Camargo AB (2017) Analytical methods for bioactive sulfur compounds in *Allium*: an integrated review and future directions. J Food Comp Analys 61:4–19

Ricroch A, Yockteng R, Brown SC, Nado S (2005) Evolution of genome size across some cultivated *Allium* species. Genome 48:511–520

Rose P, Whiteman M, Moore PK et al (2005) Bioactive S-alk(en)yl cysteine sulfoxide metabolites in the genus *Allium*: the chemistry of potential therapeutic agents. Nat Prod Rep 22:351–368

Rothberg JM, Hinz W, Rearick TM, Schultz J, Mileski W, Davey M et al (2011) An integrated semiconductor device enabling non-optical genome sequencing. Nature 475:348–352

Sang SM, Zou M, Xia Z, Lao A, Chen Z, Ho CT (2001) New spirostanol saponins from Chiense chives (*Allium tuberosum*). J Agri Food Chem 49:4780–4783

Sobolewska D, Michalska K, Podolak I, Grabowska K (2016) Steroidal saponins from the genus *Allium*. Phytochem Rev 15:1–35

Sparg SG, Light ME, Van Staden J (2004) Biological activities and distribution of plant saponins. J Ethnopharm 94:219–243

Srivastava PL, Shukla A, Kalunke RM (2018) Comprehensive metabolic and transcriptomic profiling of various tissues provide insights for saponin biosynthesis in the medicinally important *Asparagus racemosus*. Sci Rep 8:9098

Stajner D, Milic´ N, Canadanović-Brunet J, Kapor A, Stajner M, Popović BM (2006) Exploring *Allium* species as a source of potential medicinal agents. Phytother Res 20:581–584

Storsberg J, Schulz H, Keller ERJ (2003) Chemotaxonomic classification of some *Allium* wild species on the basis of their volatile sulphur compounds. J Appl Bot 77:160–162

Sun HX, Xie Y, Ye YP (2009) Advances in saponin-based adjuvants. Vaccine 27:1787–1796

Sun L, Lin S, Zhao R (2010) The saponin monomer of dwarf lilyturf tuber, DT-13, reduces human breast cancer cell adhesion and migration during hypoxia via regulation of tissue factor. Biol Pharm Bull 33:1192–1198

Teshima Y, Ikeda T, Imada K et al (2013) Identification and biological activity of antifungal saponins from shallot (*Allium cepa* L. Aggregatum group). J Agri Food Chem 61:7440–7445

Turnbull A, Galpin IJ, Collin HA (1980) Comparison of the onion plant (*Allium cepa*) and onion tissue culture. III. Feeding of ^{14}C lS1PC has two forms of isomersabeled precursor of the flavor precursor compounds. New Phytol 85:485–487

Valliyodan B, Ye H, Song L, Murphy M, Shannon JG, Nguyen HT (2017) Genetic diversity and genomic strategies for improving drought and waterlogging tolerance in soybeans. J Exp Bot 68:1835–1849

Wang C, Jogaiah S, Zhang WY, Abdelrahman M, Fang JG (2018) Spatio-temporal expression of miRNA159 family members and their GAMYB target gene during the modulation of gibberellin-induced grapevine parthenocarpy. J Exp Bot 69:3639–3650

Yoshimoto N, Saito K (2017) Biosynthesis of S-Alk(en)yl-cysteine sulfoxides in *Allium*: Retro prospective. In: De Kok LJ, Hawkesford MJ, Haneklause SH, Schnug E (eds) Sulfur metabolism in higher plants-fundamentals, environmental and agricultural aspects. Processing of International Plant Sulfur Workshop, Springer, Switzerland AG, pp 49–60

Yoshimoto N, Yabe A, Sugino Y, Murakami S, Sai-ngam N, Sumi SI, Tsuneyoshi T, Saito K (2015) Garlic γ-glutamyl transpeptidases that catalyze deglutamylation of biosynthetic intermediate of alliin. Front Plant Sci 5:758

Yu Z, Zhang T, Zhou F, Xiao X, Ding X, He H, Rang J, Quan M, Wang T, Zuo M, Xia L (2015) Anticancer Activity of Saponins from *Allium chinense* against the B16 melanoma and 4T1 breast carcinoma cell. Evid-Based Comp Alter Med 2015:725023

Yuan Y, Bayer PE, Batley J, Edwards D (2017) Improvements in genomic technologies: application to crop genomics. Trend Biotechnol 35:558–574

Zhang W, Chen G, Deng CQ (2012) Effects and mechanisms of total *Panax notoginseng* saponins on proliferation of vascular smooth muscle cells with plasma pharmacology method. J Pharm Pharmacol 64:139–145

Zhao RP, Lin SS, Yuan ST (2014) DT-13, A saponin of dwarf lilyturf tuber, exhibits anticancer activity by down-regulating C-C chemokine receptor type 5 and vascular endothelial growth factor in MDA-MB-435 cells. Chin J Nat Med 12:24–29

Zhang C, Li X, Zhan Z, Cao L, Zeng A, Chang G, Liang Y (2018) Transcriptome sequencing and metabolism analysis reveals the role of cyanidin metabolism in dark-red onion (*Allium cepa* L.) bulbs. Sci Rep 8:14109

Zhang W, Abdelrahman M, Jiu S, Guan L, Han J, Zheng T, Jia H, Song C, Fang J, Wang C (2019) VvmiR160s/VvARFs interaction and their spatio-temporal expression/cleavage products during GA-induced grape parthenocarpy. BMC Plant Biol 19:111

Chapter 8
Breeding and Genomic Approaches for Climate-Resilient Garlic

Anil Khar, Sho Hirata, Mostafa Abdelrahman, Masayoshi Shigyo and Hira Singh

Abstract Garlic (*Allium sativum* L.) has a long history of cultivation by asexual propagation. Due to its asexual nature, improvement of garlic has been limited as compared to onion. With the impending climate change, it is predicted that like all other crops, garlic cultivation will also suffer the consequences. Ninety percent of garlic is grown in Asia and increase in temperature will expose garlic to various biotic and abiotic stresses. To evolve against these stresses, quality improvement of garlic to withstand these stresses is of principal concern. Research work on creation of genetic diversity, collection of genetic resources, interspecific hybridization, and manipulation of flowering is needed through conventional techniques. Biotechnological approaches for garlic improvement through genetic transformation, marker-assisted selection, genomics-aided breeding, and other novel technologies may help in achieving higher yields under climate change scenarios. In this chapter, we have discussed various approaches and what has been done in these areas in different parts of the world to address the loss in crop yield which is likely to be caused by the biotic and abiotic stresses in the future.

Keywords Biotic resistance · Abiotic stress tolerance · Diversity evaluation · Genetic resources · Molecular breeding · Genomics · *Allium sativum* L.

A. Khar (✉) · H. Singh
Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi, India
e-mail: anil.khar@gmail.com

S. Hirata · M. Shigyo
College of Agriculture, Graduate School of Sciences and Technology for Innovation, Yamaguchi University, Yamaguchi 753-8515, Japan

M. Abdelrahman
Botany Department, Faculty of Science, Aswan University, Aswan 81528, Egypt

Arid Land Research Center, Tottori University, Tottori 680-0001, Japan

C. Kole (ed.), *Genomic Designing of Climate-Smart Vegetable Crops*,
https://doi.org/10.1007/978-3-319-97415-6_8

8.1 Introduction

Garlic, *Allium sativum* L., is an economically important diploid species of the genus *Allium* belonging to the *Alliaceae* family. This crop is considered to be one of the oldest horticultural crops. The earliest known documents indicated that garlic and onion (*A. cepa*) formed an essential part of the daily diet of several Egyptian working classes involved in the building of the pyramids, presumably to maintain and increase their strength, thereby enabling them to work harder (Moyers 1996; Mostafa et al. 2013; Abdelrahman et al. 2016, 2019). Besides this, garlic was exploited as an antiseptic to avoid gangrene during the First World War (Hedrick 1972). It is evident from the world production scenario of the last 30 years that world garlic production has increased from 5.78 to 28.16 million tons (Fig. 8.1), which is more than five times (FAOSTAT 2012; Wu et al. 2015). However, the cultivation area increased only twofold in the last 30 years (FAOSTAT 2012; Wu et al. 2015). Currently, more than 90% of garlic is produced by the Asian countries especially China and India. This crop is an asexually propagated plant with less multiplication rate, and hence having less genetic diversity. Owing to this, the development of genetically improved cultivars or creation of genetic variations through conventional breeding methods is cumbersome. Even though very few garlic genotypes flower at specific geographical regions, these genotypes exhibited sterility and location specificity which led to limit the development of new genetically improved cultivars. In the modern molecular breeding and genomic era, very less genomic studies have been conducted in garlic compared with other vegetable crops. The genome size of garlic is 15.9 GB which is marginally smaller than the onion genome (Arumuganathan and Earle 1991; Jones et al. 2004; Abdelrahman et al. 2017). Nevertheless, for the improvement of garlic, meristem culture, genetic transformation, and molecular breeding have been embarked on, but more precise research is required to develop smart climate traits in the garlic. Till date, most of the garlic cultivars released by public sector are susceptible to several viruses like onion yellow dwarf virus, leek yellow stripe virus, garlic common latent virus, shallot latent virus, and others (Sako et al. 1991; Conci et al. 1992).

Like other crops, garlic is also affected by various biotic and abiotic stresses. The changing climatic scenario may affect garlic production, but no documentary proof is there. According to Reddy et al. (2000), crop production is expected to decrease year to year even under controlled conditions due to climatic changes. Yield component of garlic is susceptible to environmental conditions (Panse et al. 2013). Thus, it needs the attention of the breeders to develop climate-smart garlic cultivars for better adaptation concerning climate changes by utilizing available genetic resources and modern biotechnological tools to ensure production sustainability. This chapter will focus on the use of breeding and genomic approaches for the climate-resilient sustenance of garlic.

The reproduction of garlic is done entirely by using its underground parts called as clove or by inflorescence vegetative top sets which are usually sterile but have high

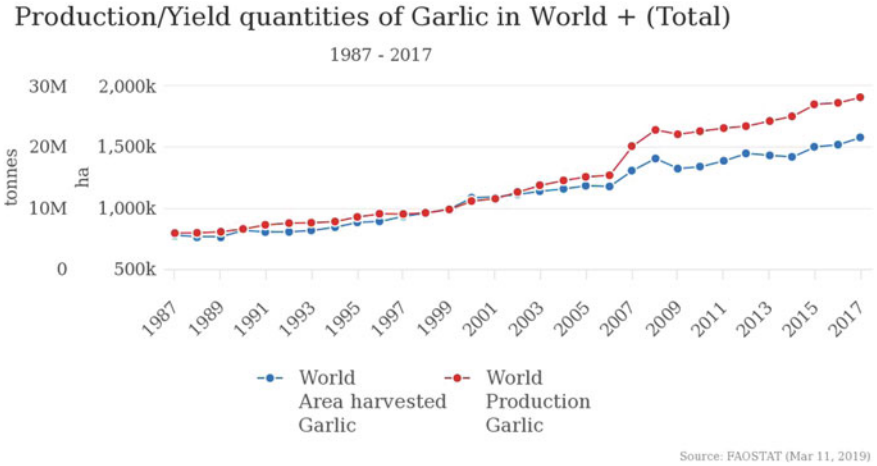

Fig. 8.1 World garlic production (million tons) and harvested area from 1987 to 2017 according to Food and Agriculture Organization (FAO http://www.fao.org)

diversity morphologically (Bradley et al. 1996; Wu et al. 2016). The asexual reproduction in garlic for many generations led to chromosomal aberrations in the form of aneuploidy and translocations/inversions which considerably limit the incidence of balanced gametes. Thus, the source of genetic variations in garlic remains the mutations (induced or random), somaclonal variations, and genetic transformation (Jones and Mann 1963; Novak 1990; Burba et al. 1993; Rubatzky and Yamaguchi 1997; Robinson 2007; Sandhu et al. 2015). The dearth of flowering and sexual reproduction in garlic limits the increase of variability that is useful for breeding for economically important traits, such as tolerance to biotic and abiotic stresses and higher yield (Kamenetsky 2007). Use of modern biotechnological tools such as molecular markers is limited and challenging due to the bigger size and complex nature of garlic genome in addition to vegetative nature of reproduction (Egea et al. 2017). Asexual reproduction could lead to narrow genomic diversity, subsequently in the clonal production since no meiosis is involved (Kamenetsky et al. 2015).

8.1.1 Climate-Smart Agronomic Trait Improvement

Development of new cultivars is dependent not only on clonal selection but also can be achieved through introduction from other garlic growing regions or environments (Jones and Mann 1963; Rubatzky and Yamaguchi 1997). Cultivation location has a significant effect on the characteristics of cultivar. Several reports revealed that changing climate could have an immense influence on flower stalk formation, taste; and a soft neck variety at a particular location might produce flower stalk when cultivated at another location (Kamenetsky et al. 2004; Kamenetsky 2007) (Table 8.1).

Table 8.1 Interaction effects of temperature and photoperiod treatments on garlic bulb characteristics in 2012

Treatment		cv. G107			cv. G025			cv. G064		
Temperature (°C)	Photoperiod (h)	Mean bulb weight (g)	Mean bulb diameter (mm)	Mean bulb height (mm)	Mean bulb weight (g)	Mean bulb diameter (mm)	Mean bulb height (mm)	Mean bulb weight (g)	Mean bulb diameter (mm)	Mean bulb height (mm)
15	8	3.47^{c}	18.09^{c}	17.32^{c}	–	–	–	–	–	–
	14	3.56^{bc}	19.10^{b}	16.80^{c}	0.87^{d}	10.73^{d}	12.44^{d}	2.27^{e}	14.93^{d}	14.60^{d}
20	8	3.64^{b}	18.95^{b}	18.02^{b}	2.00^{c}	13.60^{c}	17.26^{c}	5.25^{d}	21.00^{c}	20.10^{c}
	14	4.10^{a}	20.49^{a}	19.78^{a}	3.24^{a}	18.03^{a}	20.70^{a}	7.30^{b}	23.74^{b}	23.98^{a}
25	8	2.61^{e}	17.01^{d}	16.85^{c}	2.80^{b}	16.01^{b}	19.96^{b}	6.68^{c}	24.84^{ab}	21.84^{b}
	14	3.27^{d}	17.97^{c}	17.10^{c}	2.48^{b}	16.76^{ab}	17.42^{c}	8.24^{a}	25.29^{a}	24.07^{a}

The data are presented as the mean and 30 bulbs of each were with three replications. Different letters in the same column indicate significant differences at $P < 0.05$ (ANOVA and Duncan's multiple range test), $n = 3$

– Indicates no bulb

Source Wu et al. (2016)

8.1.2 Diversity Evaluation Study and Potential for Breeding Materials

Garlic is one of the most widely used cultivated *Allium* species and is grown in many countries at a wide range of latitudes. For centuries, this plant has been propagated clonally in various countries. It has, perhaps, caused a bottleneck effect for genetic variation (Ma et al. 2009). However, cultivated garlic or clonal lineages exhibit remarkably wide range of morphological variation in leaf number, bulb size, and structure (such as arrangement, number, and size of the cloves), floral scape length, and inflorescences (Pooler and Simon 1993a; Keller 2002; Kamenetsky et al. 2005a; Buso et al. 2008). The center of origin of garlic is opined as Central Asia region because fertile garlic was found in Kyrgyzstan, Kazakhstan, and Uzbekistan. Many researchers have studied morphological traits and molecular markers such as isozymes and DNA markers to evaluate the diversity of garlic (Pooler and Simon 1993a; Maaß and Klaas 1995; Etoh et al. 2001; Lampasona et al. 2003; Zhao et al. 2011; Jo et al. 2012; Hirata et al. 2016b). Etoh (1985) collected various garlic germplasms from worldwide including Central Asia and hypothesized that garlic evolved from fertility to sterility and from a complete bolting type to a non-bolting type through an incomplete bolting type. Moreover, Hirata et al. (2016a) demonstrated that garlic has acquired high environmental adaptability by changing the chemical composition in the bulb. Today, the evolution of garlic seems to be continuing. Other diversity studies have been carried out regarding the production level of chemicals in a set of garlic collections such as organosulfur compounds (Kamenetsky et al. 2005b; Hornickova et al. 2009; Ovesna et al. 2011; Hirata et al. 2016a) or phenolic compounds (Lu et al. 2011), which have benefits for human health. Kamenetsky et al. stated that garlic from the place of origin possesses superior traits, such as tolerance to disease and pests and better adaptation to biotic or abiotic stresses, than are seen in current cultivars. This research field could be even more important for garlic in the future.

8.1.3 Genetic Resources for Climate-Smart Genes

Albeit being utmost important bulb vegetable crops, substantial attention has not been paid to the *Allium* species for their germplasm collection and conservation since long which have led to the shortage of enough germplasm (Kamenetsky 2007). In case of garlic, not much efforts have been devoted to collect and preserve its crop wild relatives and landraces systematically which are potential source for further genetic improvement. (Rabinowitch and Zeltzer 1984; Kamenetsky 1993; Baitulin et al. 2000; Fritsch 2001; Keller and Senula 2001). The precious local gene pool is currently under severe threat of extinction, due to the rapid replacement of traditional landraces with modern cultivars (Kamenetsky et al. 2005b; Ovesna et al. 2011).

Internationally, construction of an information structure for genetic resources of garlic should be imperative in near future.

8.1.4 Abiotic Stress Tolerance

8.1.4.1 Water Stress Tolerance

Apart from genetic potential of any crop, growth and yield also depend on prevailing environmental conditions during crop development which is highly stage specific. Among all environmental aspects, water stress in the form of excess or deficiency is a challenging factor for crop production especially for vegetable crop production since these crops are of short duration requiring sufficient moisture content for their growth and development. With the continuing changes in the climate, both excess and deficit of water are the major limiting factors for vegetable production. Garlic being a shallow rooted plant exhibits significant reduction in anthocyanin, chlorophylls (a, b, and total), carotenoids, growth parameters like fresh weight of plant and root, bulb yield, quality and elevated allicin content, and increase in ion leakage under drought conditions (Bideshki et al. 2013; Diriba-Shiferaw 2016). Heavy rainfall and waterlogging conditions are also damaging to the plant growth and bulb formation (Diriba-Shiferaw 2016). In Romania, Csiszár et al. (2007) observed activities of antioxidant enzyme in three *Allium* species under drought conditions and found that after 1 week there were manipulations in the activities of enzymes related to glutathione (GR, GST) and POD in shoots linked with relative water content of leaves. Furthermore, they revealed that inducible antioxidants played great role against drought in *Allium* ancient populations. This investigation could be immensely useful for the development of new climate-smart cultivars of garlic. In Egypt, Badran (2015) conducted comparative analysis by taking four commercial garlic varieties, namely, Egaseed 2, Balady, Egaseed 1, and Sids 40 under drought conditions. On the basis of drought tolerance index, superiority measure, yield injury %, and relative performance, Egaseed 1 was found highly tolerant while Balady was found the highly sensitive variety. Further, he used five inter-simple sequence repeat (ISSR) primers and observed 50.83% of mean polymorphism and only three primers (HB08, HB11, and 44B) showed unique bands. The ISSR marker analysis could be exploited to distinguish garlic cultivars across any breeding program.

8.1.4.2 Salinity Tolerance

Salinity is an important stress which affects the crop yield worldwide. Not much research work has been done on this area in garlic. With the changing climatic scenario, knowledge about the salt stress levels of garlic cultivars will be a viable option to work toward identification and development of salt-tolerant varieties. Silenzi et al. (1985) suggested that salinity (0.96–5.40 dS m^{-1}) delays sprouting but has no effect

on the final amount of sprouting. Mangal et al. (1990) estimated that in garlic, 50% yield reduction occurs at 5.60–7.80 dS m^{-1}, depending upon the genotype. They also estimated that if soil salinity exceeds 1.70 dS m^{-1}, the mean garlic yield declined by 1.68% per unit increase in soil salinity. Francois (1994) indicated a tolerance threshold of 3.9 dS m^{-1} and a yield decline of 14.3% for each unit increase in salinity above the threshold. Although salt tolerance threshold of garlic was slightly higher than most vegetable crops, yields drop rapidly once the soil salinity values exceed the threshold (Maas and Hoffman 1977).

8.1.4.3 Thermal Stress and Photoperiod

Thermal stress is one of the major abiotic stresses which restricts germination, plant growth, metabolism, and productivity worldwide. The processes starting from seed germination to senescence of plant include several biochemical reactions and enzyme activities that are highly sensitive to temperature. Response of crop plants to temperature depends upon the duration and the degree of the temperature. Temperature stress is now a foremost apprehension for the crop breeders for sustaining crop productivity.

The documented studies revealed that environmental factors such as temperature, photoperiod, etc. play immense role in Allium vegetative and reproductive growth and development (Takagi 1990; Pooler and Simon 1993b; Brewster 1994; Kamenetsky and Rabinowitch 2002; Etoh and Simon 2002; Kamenetsky et al. 2004). In garlic, the transition of the apical meristem from a vegetative to a reproductive state occurs during the active growing phase (Kamenetsky and Rabinowitch 2001). Low temperatures promote floral development, and long photoperiod is essential for floral scape elongation (Takagi 1990). Kamenetsky et al. (2004) observed that high temperature with long photoperiod enhanced the translocation of reserves to the cloves, and the degeneration of the developing inflorescence. It was further concluded that in bolting garlic genotypes, manipulation of the environment, both before and after planting, can regulate the development of flowers and regain fertility. Recently, Wu et al. (2016) concluded that higher endogenous phytohormone (especially GA) and MeJA levels are beneficial for garlic bolting and bulbing which varied with various treatment combinations of photoperiod and temperature. Son et al. (2012) studied response of garlic to cold stress and isolated 15 upregulated and 4 downregulated cold-responsive genes. These cold-responsive (CR) genes can be manipulated to overcome frost damage in garlic during its hibernation in the field conditions.

8.1.5 Biotic Stress Tolerance

8.1.5.1 Insect-Pest and Disease Resistance

Under changing climate scenario, there are many documented reports of damage caused by the abrupt spread of insect-pests and diseases in field and horticultural

crops. It is a strong indication of climate change that is manipulating the intensity, distribution, and incidence of crop pests and diseases (Lamichhane et al. 2015). Garlic is prone to many diseases such as basal rot (*Fusarium culmorum*) (Mishra et al. 2014), white rot (*Sclerotium cepivorum*) (Zewde et al. 2007), downy mildew (*Peronospora destructor*) (Schwartz 2004), Botrytis rot (*Botrytis porri*) (Wu et al. 2012), Penicillium decay (*Penicillium hirsutum*) (Dugan 2007), and nematodes (Insunza and Valenzuela 1995). Most of the major garlic diseases are soil-borne, so proper site assessment and yearly rotations are crucial in maintaining a healthy garden of garlic.

8.1.5.2 White Rot

This disease is caused by fungus, *Sclerotium cepivorum*, which is one of the devastating global garlic diseases (Schwartz and Mohan 1995; Nabulsi et al. 2001). In Syria, Al-Safadi et al. (2000) started mutation breeding of garlic to get mutants resistant to white rot using gamma radiation and successfully achieved resistant mutants. Furthermore, Nabulsi et al. (2001) used random amplified polymorphic DNA (RAPD) analysis to elucidate molecular diversity among eight mutants of garlic through 13 random primers. Twelve primers showed polymorphism in amplification products and further highly resistant mutants were quite distant from the control with low correlation coefficients. The pattern of bands displayed by primer OPB-15 (GGAGGGTGTT) with highly resistant mutant could be exploited as genetic marker for further garlic breeding program

8.1.5.3 Blue Mold Disease

This garlic disease is caused by many *Penicillium* species and has been attributed to significant annual crop losses. Symptoms include stunted and chlorotic plants with withered leaves and reduced bulb size (Valdez et al. 2006). In Argentina, Cavagnaro et al. (2005a, b) evaluated garlic accessions against *Penicillium hirsutum* and found significant differences in the accessions. Accessions Castano and Morado were most resistant and it was further observed that there was a low correlation ($r = 0.17$) between allicin content and tolerance against this disease, indicating that allicin is not the main factor involved in the resistance against *P. hirsutum*.

8.1.5.4 Purple Blotch and Stemphylium Blight

The causal organism of purple blotch infection is *Alternaria porri* while Stemphylium blight is caused by *Stemphylium vesicarium*. Often both the diseases appear together and exhibit a complex of symptoms. There are less sources of available host plants that exhibit resistance against purple blotch naturally. Genetic engineering or transgenic could be an alternate toward purple blotch resistance (Eady et al. 2003) but consumer

non-preference due to ethical/biosafety issues have not allowed the transgenics to grow on commercial level. In this situation, host resistance breeding could be the most efficient way to control purple blotch disease (Nanda et al. 2016). Rout et al. (2016) isolated and characterized a PR5 gene, designated as *AsPR5*, induced in response to *Fusarium oxysporum* f. sp. *cepae* (FOC) infection in garlic. Their results suggest that, besides antifungal activities, *AsPR5* also plays a significant role in activating multiple defense pathways for enhancing stress resistance.

8.2 Genomic Approaches for Climate-Smart Garlic

Changing climate is a global phenomenon, and it is a continuous process since long with a long-term effect on agriculture productivity and food security. Thus, managing such changes is now demanding the attention of the agri-scientists and policymakers (Raza et al. 2019). Furthermore, it is predicted that future agriculture evolution will be designed by its response to climate change (Zilberman et al. 2018). For the adoption and development of climate-smart garlic cultivars, subsequent strategies are needed to combat environmental stresses.

8.2.1 Interspecific Hybridization

With the inception of agriculture, the genus *Allium* has played a significant role as vegetable and spice crop with a long history of cultivation of various *Allium* species either in cultivated or semi-cultivated form. This genus has immense genetic diversity in the form of bulb species, leaf shape, sexual or asexual reproduction, and tolerance to various biotic and abiotic stresses. To impart resistance or tolerance against various abiotic and biotic stresses, interspecific hybridization plays an important role to introgress alien genes into domesticated cultivated species. Among the *Allium* species, several attempts have been made for the improvement of onion (*Allium cepa*) (Keller et al. 1996; Peffley and Hou 2000) but in case of garlic not much efforts have been put due to its sterile nature and asexual mode of reproduction. There are very few scientific reports on interspecific hybridization between leek (*Allium ampeloprasum* L.) and garlic to introduce fertility and disease resistance into garlic (Sugimoto et al. 1991; Yanagino et al. 2003). The *Allium* species *A. ampeloprasum* is mainly used as a leafy vegetable, propagated through seeds and has the possibility to develop bulb in the summer season; on the other side, garlic is used as bulb spice crop and vegetatively propagated. Nevertheless, taxonomically garlic and leek are narrowly associated and both are grouped into the subgenus *Allium*. Porter and Jones (1932) documented that leek has some disease resistance genes while garlic has not. To create genetic variation and new *Allium* crops, interspecific hybridization between *A. sativum* and *A. ampeloprasum* could be the alternative option for the climate-smart cultivar development in garlic crop. In Japan, Yanagino et al. (2003)

Table 8.2 Foliage and bulb characteristics of leek, garlic and the interspecific hybrid ± indicates standard deviation

	No. of plants examined	Length of the longest leaf (cm)	No. of plants which formed a bulb	Bulb weight (g)	No. of cloves per bulb
Leek	9	99.7 ± 9.8	5	65.8 ± 19.4	1.8 ± 0.4
Garlic	16	97.6 ± 9.7	16	45.8 ± 12.6	9.3 ± 0.9
Hybrid	13	113.6 ± 9.0	13	70.1 ± 13.1	4.0 ± 0.6

Source Yanagino et al. (2003)

produced interspecific hybrids between leek and garlic successfully through ovary culture. They used some identified fertile clones of garlic as male parent through ovary culture. The hybridity of the interspecific cross was validated through morphological observations cytologically ($2n = 3x = 24$) and molecular analysis using RAPD markers. Further, their results revealed that the hybrid exhibited intermediate characteristics between the parental species such as growth, foliage, and bigger bulb size and garlic odor. Success and results of this interspecific hybrid indicated that this could have the potential to be a new crop having diverse genetic makeup and wide adaptability (Table 8.2).

8.3 Biotechnological Approaches

Being a vegetatively propagated crop, selection of diverse clones is one of the conventional approaches for garlic improvement. These diverse clones are developed through natural mutations or occasional production of sexual progenies (Etoh and Simon 2002; Havey and Ahn 2016). Landraces and local farmer developed cultivars are the significant source of various climate-smart genes for the various stresses. With the advent of biotechnological approaches, improvement of garlic can be initiated by utilizing a plethora of non-conventional approaches.

8.3.1 Genetics and Genomics Strategies In Vitro Culture Based Methods

8.3.1.1 Genetic Transformation

Foreign gene transfer to plants is becoming a routine technique for many important crop species. The presence of efficient methods of genetic transformation—*Agrobacterium*-mediated transformation or direct gene transfer by particle bombardment (Songstad et al. 1995)—is of considerable importance for the improvement of

modern crops. *Agrobacterium tumefaciens* is routinely utilized in gene transfer in case of dicotyledonous plants. Monocotyledonous plants were thought to be recalcitrant to this technology as they were outside the host range of the bacterium. However, recently transgenic plants have been obtained in some monocotyledonous spp. using specific *Agrobacterium* strains (Arencibia et al. 1998; Liu et al. 1998; Khanna and Raina 1999). Therefore, the monocotyledonous nature of species no longer prevents the application of *Agrobacterium*-mediated techniques for the transfer of genes to these species as soon as the methodological parameters are optimized (Hiei et al. 1997).

Genetic transformation in garlic is of utmost importance because of its sexual sterility. Due to difficulties of inducing flowering, breeding programs have been limited to clonal selection and production of virus-free stocks via meristem culture. Although, tissue culture is a useful technique for producing virus-free garlic seedlings, the propagation rate of virus-free plantlets is very low. And the process is laborious and time-consuming. Since no other methods of gene transfer exist, the genetic transformation may be a promising tool. Transformation also holds the key for the improvement of garlic toward biotic and abiotic stresses. It is possible to use the genetic transformation for the production of transgenic garlic with the desired characteristics. Unfortunately, both onion and garlic have proved to be recalcitrant to genetic transformation and plant regeneration (Eady et al. 1996).

Introduction of alien DNA into plant cells can be achieved by using the bacterium *A. tumefaciens* (indirect method) or biolistic method (direct method) as a vehicle. In biolistic approaches, Barandiaran et al. (1998) were first to attempt garlic transformation using biolistic approach to transfer and detect the transient expression of *uid*A gene into different garlic tissues, including regenerable calli using nuclease inhibitor aurintricarboxylic acid. Later, Ferrer et al. (2000) introduced, by biolistic method, reporter gene *uid*A and selection gene *bar* in leaf tissue, basal plate disc, and embryogenic calli and reported maximum expression of *uid*A gene in calli and leaves. Sawahel (2002) showed that biolistic transformation could lead to the expression and stable integration of a DNA fragment into immature cloves, whereas Park et al. (2002) established an effective biolistic transformation procedure for obtaining chlorsulfuron-resistant transgenic plants by incorporating *ALS* gene coding for acetolactate synthase. Later Robledo-Paz et al. (2004) were able to introduce DNA into embryogenic garlic callus and produce stably transformed garlic plants.

Use of *Agrobacterium*-mediated transformation was initiated by Kondo et al. (2000) who were able to develop a stable transformation system of garlic using highly regenerative calli. Zheng et al. (2004) developed a reliable transformation system to produce garlic plants containing *Bt* resistance genes which conferred resistance to beet armyworm (*Spodoptera exigua*). Khar et al. (2005) studied the transitory expression of the reporter gene *gusA* in two garlic cultivars after infecting them with *A. tumefaciens*, whereas Eady et al. (2005) recovered transgenic garlic plants from immature embryos using *A. tumefaciens* containing the vector pBIN *mgfp-ER* which includes the modified *gfp* reporter gene and the *npt*II selectable marker gene. Later, Kenel et al. (2010) developed a method for garlic transformation from immature leaves containing the *mgfp*-ER reporter gene and *hpt* selectable gene. Regenerated

transgenic plants survived in the glasshouse and matured into healthy plants. Ahn et al. (2013) were successful in increasing the stable transformation efficiency (up to 10.6%) by using a two-step selection involving hygromycin resistance and green fluorescent protein (GFP) expression. Transgenic garlic plants stably integrated and expressed the phosphinothricin acetyltransferase (*PAT*) gene, and they demonstrated that transgenic plants conferred herbicide resistance, while nontransgenic plants and weeds died. Quality and yield of garlic are diminished due to white rot disease (*Sclerotium cepivorum* Berk). Fortiz et al. (2013) developed a transformation protocol to introduce tobacco chitinase and glucanase genes into garlic embryogenic calli using *A. tumefaciens* and were able to develop transformed plants which were not completely resistant but exhibited a delay in fungal infection.

8.3.1.2 Meristem Tip Culture

Vegetative propagation leads to accumulation of viruses, and it is well established that garlic is susceptible to accumulation of a complex of viruses, notably members of the genera *Potyvirus*, *Carlavirus*, *Allexivirus*, and *Potexvirus* (King et al. 2012). Losses in yield and deterioration in quality are the well-established problems associated with virus infections. Control of these viruses is problematic and involves the production of virus-free plants by meristem tip culture and subsequent multiplication of plants under aphid-free conditions. Production of virus-free garlic plants has been attained through shoot tip culture (Peña-Iglesias and Ayuso 1982), scape tip culture (Ma et al. 1994), small inflorescence bulbils culture (Ebi et al. 2000), "stem disc dome culture" (Ayabe and Sumi 2001), and meristem tip culture (Wei and We 1992). Attempts to obtain virus-free garlic through thermotherapy (Conci and Nome 1991; Ucman et al. 1998), a combination of meristem tip culture and thermotherapy (Robert et al. 1998) and use of chemotherapy (Ramírez-Malagón et al. 2006) have been reported. It has also been concluded that virus-free garlic yields better and has better quality than the virus-infected plants (Ramírez-Malagón et al. 2006). New methods of development of virus-free garlic through cryotherapy of shoot tips (Vieira et al. 2015) and root tip culture (Haque and Hattori 2017) have been reported. Although many papers on development of virus-free garlic through various methods have been documented, field performance and a protocol for production of these virus-free garlic plants for commercial production are still lacking.

8.3.1.3 Somaclonal Variations

Since all commercially grown garlic cultivars are sterile, they can only be vegetatively propagated; this habit of garlic has restricted the development of new, improved cultivars through the utilization of plant breeding approaches. To create genetic variation and new forms of the crop, somaclonal variants produced during long-term tissue culture could be a potential option for garlic (Al-Zahim et al. 1999). In vitro regeneration of plants through callus culture has been documented since long (Kehr

and Schäffer 1976; Abo El-Nil 1977; Xue et al. 1991) but achievement in this aspect is limited. Novák (1980) observed such variations phenotypically and cytologically. Furthermore, higher bulb weight was noticed in some somaclones compared to the parental ones (Vidal et al. 1993).

8.3.2 Molecular Breeding

8.3.2.1 Molecular Markers

Molecular markers are being used extensively for determination of genetic diversity because of their neutral nature, reproducibility of results across labs, and no environmental effect on their expression. Genetic diversity of garlic has been assessed by isozymes (Pooler and Simon 1993a), random amplified polymorphic DNA (RAPD) markers (Ipek et al. 2003; Khar et al. 2008; Maaß and Klaas 1995), inter-simple sequence repeat (ISSR) markers (Jabbes et al. 2011), combination of RAPD and ISSR markers (Shaaf et al. 2014), sequence-related amplified polymorphism (SRAP) markers (Chen et al. 2013), amplified fragment length polymorphism (AFLP) markers (Volk et al. 2004; García-Lampasona et al. 2012), and locus-specific markers (Ipek et al. 2008). Estimation of garlic diversity using microsatellite markers was first reported by Ma et al. (2009) wherein they were able to develop a simple sequence repeat (SSR) enriched library and finally reported eight SSRs for diversity estimation. The same eight SSRs were used by Zhao et al. (2011) for molecular genetic diversity studies, population structure analysis, and core collection estimation followed by Jo et al. (2012) who classified genetic variation in 120 accessions from five different countries using the seven primers out of the same eight SSRs reported earlier. Cunha et al. (2012) reported a new set of 16 SSR markers using (CT)8- and (GT)8-enriched library and found 10 markers to be polymorphic, whereas Chen et al. (2014) used the same set of markers and found eight to be polymorphic. Khar (2012) used 99 SSRs and reported 18 polymorphic SSR for estimation of genetic diversity in garlic. Recently, Cunha et al. (2014) were able to assess the genetic diversity and population structure of Brazilian accessions using 17 SSR markers developed by Ma et al. (2009) and Cunha et al. (2012) (Fig. 8.2).

8.3.2.2 Genetic Linkage Maps

Genetic linkage maps are powerful tools for localization of genes, understanding the genetic basis of complex traits, marker-assisted breeding, and map-based cloning of important genes. Development of a genetic linkage map will enhance garlic improvement by allowing marker-assisted selection (MAS) and identification of genes that control economically important traits. The first genetic map of garlic (Zewdie et al. 2005) was developed using 37 markers forming nine linkage groups, and a male

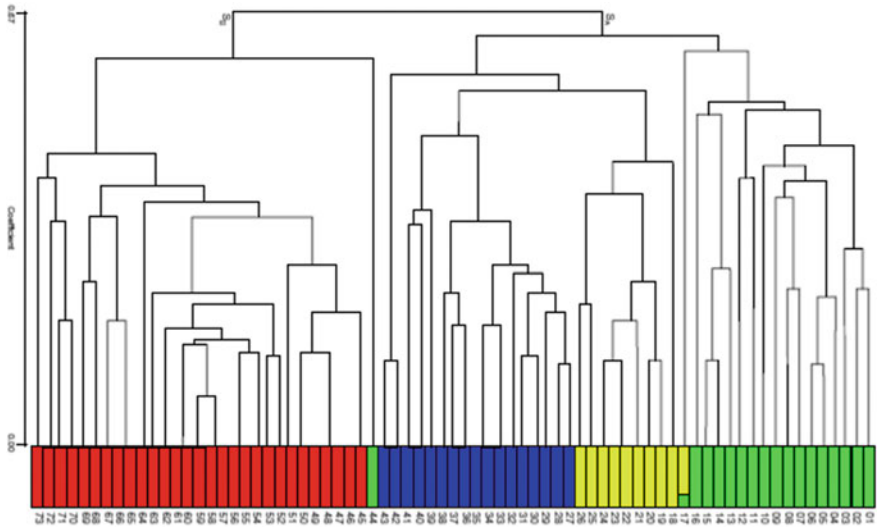

Fig. 8.2 UPGMA dendrogram based on Rogers-W genetic distance for 73 garlic accessions with a cophenetic correlation coefficient of 0.92. The major clusters are highlighted with the corresponding color of subgroups identified by the model-based clustering technique: SA and SB ($K = 2$); and S_1 (green), S_2 (yellow), S_3 (blue), and S_4 (red) ($K = 4$). *Source* Cunha et al. (2014)

fertility locus was placed on the map. This was followed by the development of the first low-density genetic map (Fig. 8.3) based on AFLP markers (Ipek et al. 2005).

8.3.3 Genomics-Aided Breeding

For crop improvement, omics approaches offer potential resources to study biological functions of any genetic information (Stinchcombe and Hoekstra 2008) which also help to unravel meaningful biological regulatory networks (Keurentjes et al. 2008). Almost all important crop improvement breeding programs include genomics approaches combined with conventional breeding to shorten the time and to evaluate the elite germplasm (Bevan and Waugh 2007). Such modern biotechnological tools assist considerably to develop climate-smart crops with higher yield potential under climate change scenario (Roy et al. 2011).

8.3.3.1 Genomics-Based Markers

It could be said that molecular plant breeding is a critical and efficient approach to augmenting crop production under several abiotic and biotic stresses (Wani et al. 2018). Garlic is a diploid ($2n = 2x = 16$) *Allium* species having 16.2 GB genome

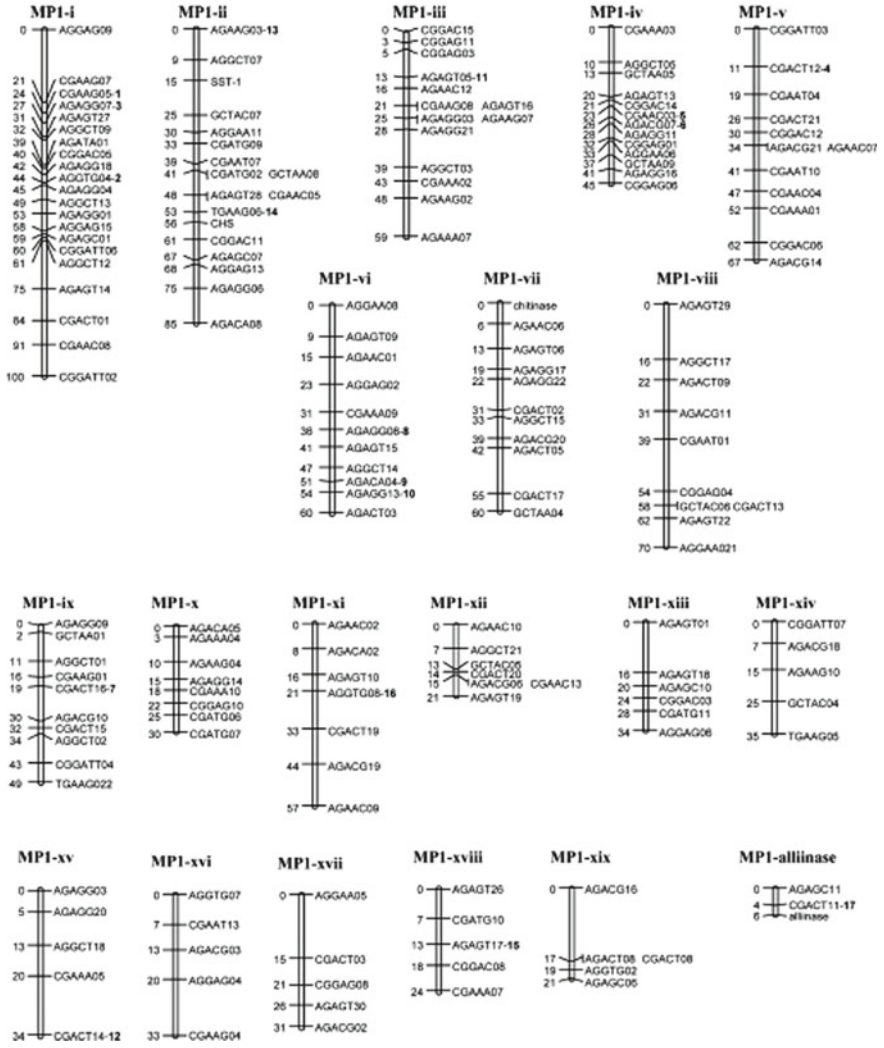

Fig. 8.3 Linkage map in the genetic map of *Allium sativum*, based on the MP1 family. Map distance is in centiMorgans. *Numbers in boldface* are common markers segregating in both MP1 and MP2. *Source* Ipek et al. (2005)

per 1C nucleus size (Bennett and Leitch 2012; Havey and Ahn 2016). With such a large genome size, sequencing of expressed regions is an effective method to detect various genes and escaping of repetitive DNA issues (Newman et al.1994; Kuhl et al. 2004; Gore et al. 2009; Havey and Ahn 2016). Genetic diversity and polymorphism in garlic have been studied through various molecular markers such as RAPD, SSR, AFLP, and insertions–deletions (Maaß and Klaas 1995; Ipek et al. 2005; Ovesná et al. 2014; DaCunha et al. 2014; Ipek et al. 2015; Wang et al. 2016).

However, genetic diversity studies in garlic are still challenging (Kim et al. 2009). Till 2009, there was no available tool or integrated database for garlic which could give the information about annotations and expressed sequence tags (ESTs). Later on, Kim et al. (2009) developed the GarlicESTdb using a pipeline system which offers access to all garlic EST resources and comprehensive database containing information about the cluster, annotation, protein domain, pathway, tandem repeat, single nucleotide polymorphism (SNP), etc. To carry forward this genomics research in garlic, polymorphism among garlic germplasm has been revealed through transcriptome sequencing in expressed regions which is helpful for diversity analysis and genetic map development (Kuhl et al. 2004; Martin et al. 2005; Gore et al. 2009; Duangjit et al. 2013; Ipek et al. 2015). Use of molecular markers like AFLPs, SSRs, and SNPs have been identified (Ipek et al. 2005, 2015; Ma et al. 2009; Zewdie et al. 2005; Zhao et al. 2011). Until now, similar appearance and phenotypic plasticity of garlic varieties hinder their morphological classification. Molecular studies are challenging, due to the large and expected complex genome of this species, with asexual reproduction. Classical molecular markers, like isozymes, RAPD, SSR, or AFLP, are not convenient to generate germplasm core collections for this species. The recent emergence of high-throughput genotyping-by-sequencing (GBS) approaches, DArTseq technology, allows to overcome such limitations to characterize and protect genetic diversity. Therefore, such technology can be used in garlic to: (i) assess genetic diversity and structure of a large garlic germplasm bank; (ii) create a core collection; (iii) relate genotype to agronomical features; and (iv) describe a cost-effective method to manage genetic diversity in garlic germplasm banks.

8.3.3.2 Genome-Wide Association Studies (GWAS) for Stress Tolerance

To understand the full set of genetic variants in crop cultivars and to identify allelic variants linked with any specific trait, genome-wide association studies (GWAS) is one of the powerful genomic technologies (Manolio 2010). GWAS has been conducted to reveal the genetic background responsible for the resistance at the genetic level under climate change (Mousavi-Derazmahalleh et al. 2018). In plants, GWAS has widespread applications related to biotic and abiotic stresses (Lafarge et al. 2017; Thoen et al. 2017). The first work of high-throughput garlic genotyping was done by Egea et al. (2017). They reduced significantly the garlic germplasm bank size, identifying redundant accessions and thus generating a unique (non-redundant) core collection, with the consequent reduction in space and maintenance expenses. They further suggested that DArTseq analysis is a cheaper method to perform genotyping-by-sequencing and genetic diversity analyses of garlic having gigantic, complex, and without a reference genome, and gave reliable results, according to genotype and their geographical origin. With this study, it would be easy for the breeders to select genotype from characterized core collection for better adaptability against various biotic and abiotic stresses under changing climate and global warming.

8.3.3.3 Next-Generation Sequencing

Recently, transcriptome profile of garlic buds, using Illumina sequencing technology, has been achieved. A total of 127,933 unigenes have been generated, annotated functionally, and analyzed about their gene ontology and metabolic pathways. Genes encoding enzymes involved in sulfur assimilation pathway were discovered which will provide the foundation for the research on gene expression, genomics, and functional genomics in *Allium sativum* and closely related species (Sun et al. 2012). Sun et al. (2013) studied the transcriptional profiling between the dormant and sprouting garlic shoot apex and observed that the expression of 22,836 unigenes was increased by more than two fold in sprouting garlic shoot apex as compared with dormant shoot apex. Based on the findings, they postulated that differential expression of genes, such as *ENHYDROUS*, *DAG1*, *DAM*, *DTH8*, play a critical role in shoot apex sprouting and may serve as candidate genes for sprouting regulation in *Allium* species. Studies on the molecular characterization of nuclear binding site encoding resistance genes and induction analysis of a putative candidate gene linked to Fusarium basal rot resistance in *Allium sativum* (Rout et al. 2014) led to identification of 28 *AsRGA* (*A. sativum* resistance gene analogs) sequences from a resistant garlic genotype CBT-As153 that can form the basis towards *Fusarium* basal rot resistance. The identified *AsRGAs* can act as a valuable resource toward the development of resistance gene analog-based molecular markers for genetic mapping in garlic that will pave the way toward cloning of novel *R*-gene against *F. oxysporum* f. sp. *cepae*.

8.4 Future Perspectives

Garlic is an essential horticultural crop, because of its nutritional and medicinal properties. In addition to fresh garlic consumption, the production of processed and dried garlic products for use as dietary health-food supplements and food processing is an important industry. Garlic breeding has been constrained by the absence of adequate methods to generate variation in the existing germplasm due to sexual sterility of garlic. In addition, flower development and fertility of garlic plants are strongly regulated by environmental conditions, and therefore the garlic seed production in various climatic zones is challenging and needs further studies. Currently, most progress in achieving genetic improvement in garlic has been through clonal selection, but standardization of biotechnological methods to induce variations still needed further efforts. Restoring fertility in garlic provides new genetic possibilities for breeding purposes. Garlic breeding improvement through using modern techniques to increase variation like mutagenesis, sexual hybridization, genetic transformation, and the current developments in florogenesis can be successfully implemented, which might help to increase the genetic variability, opening new avenues for the breeding of this important crop. For instance, biolistic and *Agrobacterium* gene transfer systems were improved in the last years and the first transgenic garlic lines have already been produced. Moreover, garlic embryogenic cell suspensions have been described.

Although large steps forward have been made at the fundamental level (e.g., garlic genome organization, genetic transformation, florogenesis, and embryogenic cell suspension development), it is also clear that there are still large gaps present in our knowledge. Few accessions have been developed to carry out interspecific gene introgression, and genetic linkage maps have begun to be developed with few significant loci placed on approximate locations of the genome. In addition, molecular markers like RAPD, SSR have been developed for diversity studies. All these developments in garlic breeding system innovation show that there are good opportunities for the production of improved garlic cultivars. If results of these researches are systematically interpreted and applied in garlic breeding, production, and storage, garlic can become highly remunerative.

References

Abdelrahman M, Abdel-Motaal F, El-Sayed M, Jogaiah S, Shigyo M, Ito S, Tran LS (2016) Dissection of *Trichoderma longibrachiatum* induced-defense in onion (*Allium cepa* L.) against *Fusariumoxysporum* f. sp. cepae by target metabolite profiling. Plant Sci 246: 128e138

Abdelrahman M, El-Sayed M, Sato S, Hirakawa H, Ito SI, Tanaka K, Mine Y, Sugiyama N, Suzuki M, Yamauchi N, Shigyo M (2017) RNA-sequencing-based transcriptome and biochemical analyses of steroidal saponin pathway in a complete set of *Allium fistulosum-A. cepa* monosomic addition lines. PLoS One 12:e0181784

Abdelrahman M, Hirata S, Sawada Y, Hirai MY, Sato S, Hirakawa H, Mine Y, Tanaka K, Shigyo M (2019) Widely targeted metabolome and transcriptome landscapes of *Allium fistulosum–A. cepa* chromosome addition lines revealed a flavonoid hot spot on chromosome 5A. Sci Rep 9: 3541

Abo El-Nil MM (1977) Organogenesis and embryogenesis in callus culture of garlic (*Allium sativum* L.). Plant Sci Lett 9:259–264

Ahn YK, Yoon MK and Jeon JS (2013) Development of an efficient *Agrobacterium*-mediated transformation system and production of herbicide-resistant transgenic plants in garlic (*Allium sativum* L.). Mol Cells 36(2):158–162

Al-Safadi B, Mir AN and Arabi MIE (2000) Improvement of garlic (Allium sativum L.) resistance to white rot and storability using gamma irradiation induced mutations. J Genet Breed 54(3):175–182

Al-Zahim MA, Ford-Lloyd BV and Newbury HJ (1999) Detection of somaclonal variation in garlic (*Allium sativum* L.) using RAPD and cytological analysis. Plant Cell Rep 18(6):473–477

Arencibia AD, Carmona ER, Teller P, Chan MT, Yu SM, LE Trujilo S, Oamas P (1988) An efficient protocol for sugarcane (*Saccharrum* spp. L.) transformation mediated by *Agrobacterium tumefaciens*. Transgen Res 7:213–222

Arumuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9(3):208–218

Ayabe M, Sumi S (2001) A novel and efficient tissue culture method—"stem-disc dome culture"—for producing virus-free garlic (*Allium sativum* L.). Plant Cell Rep 20:503–507

Badran AE (2015) Comparative analysis of some garlic varieties under drought stress conditions. J Agri Sci 7(10):271

Baitulin IO, Agafonova G, Rabinowitch HD, Kamenetsky R (2000) Creation of gene bank of Central Asian species of the genus *Allium* L., their biology and economic potential (in Russian). In: Granovsky EI, Fain EE (eds) State and perspectives of scientific collaboration Kazakhstan-Israel. Kazakhstan, Almaty, pp 87–94

Barandiaran X, di Pietro A, Martin J, Di Pietro A (1998) Biolistic transfer and expression of a *uid*A reporter gene in different tissues of *Allium sativum* L. Plant Cell Rep 17(9):737–741
Bennett MD, Leitch IJ (2012) Plant DNA C-values database (release 6.0). 27 Oct 2015. http://www.kew.org/cvalues/
Bevan M, Waugh R (2007) Applying plant genomics to crop improvement. BioMed Central, London, UK
Bideshki A, Arvin MJ, Darini M (2013) Interactive effects of Indole-3-butyric acid (IBA) and salicylic acid (SA) on growth parameters, bulb yield and allicin contents of garlic (*Allium sativum*) under drought stress in field. Intl J Agron Plant Product 4(2):271–279
Bradley KF, Rieger MA, Collins GG (1996) Classification of Australian garlic cultivars by DNA fingerprinting. Aust J Exp Agri 36:613–618
Brewster JL (1994) Onions and other vegetable Alliums. CAB International, Wallingford, UK
Burba JL, Casali VW, Buteler MI (1993) Intensidad de la dormicioncomoparametrofisiologico para agruparcultivares de ajo (*Allium sativum* L.). Hort Argen 12(32):47–52
Buso GS, Paiva MR, Torres AC, Resende FV, Ferreira MA, Buso JA, Dusi AN (2008) Genetic diversity studies of Brazilian garlic cultivars and quality control of garlic-clover production. Genet Mol Res 7:534–541
Cavagnaro PF, Camargo A, Piccolo RJ, Lampasona SG, Burba JL, Masuelli RW (2005a) Resistance to *Penicillium hirsutum* Dierckx in garlic accessions. Eur J Plant Pathol 112(2):195–199
Cavagnaro PF, Senalik D, Galmarini CR, Simon PW (2005b) Correlation of pungency, thiosulfinates, antiplatelet activity and total soluble solids in two garlic families. Annu Conf HortScience 40(4):1019
Chen S, Zhou J, Chen Q, Chang Y, Du J, Meng H (2013) Analysis of the genetic diversity of garlic (*Allium sativum* L.) germplasm by SRAP. Biochem Syst Ecol 50:139–146
Chen S, Chen W, Shen X, Yang Y, Qi F, Liu Y, Meng H (2014) Analysis of the genetic diversity of garlic (*Allium sativum*) by simple sequence repeat and inter simple sequence repeat analysis and agro-morphological traits. Biochem Syst Ecol 55:260–267
Conci V, Nome S (1991) Virus free garlic (*Allium sativum* L.) plants obtained by thermotherapy and meristem-tip culture. J Phytopathol 132:186–192
Conci V, Nome SF, Milne RG (1992) Filamentous viruses of garlic in Argentina. Plant Dis 76:594–596
Csiszár J, Lantos E, Tari I, Madosa E, Wodala B, Vashegy A, Horváth F, Pécsváradi A, Szabó M, Bartha B, Gallé Á (2007) Antioxidant enzyme activities in Allium species and their cultivars under water stress. Plant Soil Environ 53(12):517
Cunha CP, Hoogerheide ESS, Zucchi MI, Monteiro M, Pinheiro JB (2012) New microsatellite markers for garlic *Allium sativum* (Alliaceae). Amer J Bot 99:17–19
Cunha CP, Resende FV, Zucchi MI, Pinheiro JB (2014) SSR-based genetic diversity and structure of garlic accessions from Brazil. Genetica 142:419–431
Diriba-Shiferaw G (2016) Review of management strategies of constraints in garlic (*Allium sativum* L.) production. J Agri Sci–Sri Lanka 11(3):186–207
Duangjit J, Bohanec B, Chan AP, Town CD, Havey MJ (2013) Transcriptome sequencing to produce SNP-based genetic maps of onion. Theor Appl Genet 126:2093–2101. https://doi.org/10.1007/s00122-013-2121-x
Dugan FM (2007) Diseases and disease management in seed garlic: problems and prospects. Amer J Plant Sci Bioctechnol. 1:47–51
Eady CC, Lister CE, Suo Y, Schaper D (1996) Transient expression of *uid*A constructs in in vitro onion (*Allium cepa* L.) cultures following particle bombardment and *Agrobacterium*-mediated DNA delivery. Plant Cell Rep 15:958–962
Eady C, Davis S, Farrant J, Reader J, Kenel F (2003) *Agrobacterium tumefaciens*-mediated transformation and regeneration of herbicide resistant onion (*Allium cepa* L.) plants. Ann Appl Biol 142:213–217
Eady CC, Davis S, Catanach A, Kenel F, Hunger S (2005) *Agrobacterium tumefaciens*-mediated transformation of leek (*Allium porrum*) and garlic (*Allium sativum*). Plant Cell Rep 24:209–215

Ebi M, Kasai N, Masuda K (2000) Small inflorescence bulbils are best for micropropagation and virus elimination in garlic. HortScience 35:735–737
Egea LA, Mérida-García R, Kilian A, Hernandez P, Dorado G (2017) Assessment of geneticdiversity and structure of largegarlic (*Allium sativum*) germplasmbank by diversityarraystechnology "Genotyping-by-Sequencing" platform (DArTseq). Front Genet 8:98. https://doi.org/10.3389/fgene.2017.00098
Etoh T (1985) Studies on the sterility in garlic, *Allium sativum* L. Mem Fac Agri Kagoshima Univ 21:77–132
Etoh T, Simon PW (2002) Diversity, fertility and seed production of garlic. In: Rabinowitch HD, Currah L (eds) *Allium* crop science: recent advances. CABI, New York, pp 101–107
Etoh T, Watanabe H, Iwai S (2001) RAPD variation of garlic clones in the center of origin and the westernmost area of distribution. Mem Fac Agr Kagoshima Univ 37:21–27
FAOSTAT (2012) http://faostat3.fao.org/faostat-gateway/go/to/download/Q/QC/E
Ferrer E, Linares C, Gonzalez JM (2000) Efficient transient expression of the beta-glucuronidase reporter gene in garlic (*Allium sativum* L.). Agronomie 20:869–874
Fortiz EL, Paz AR, Espinosa1 MAG, Mascorro-Gallardo JM, Rangel EE (2013) Genetic transformation of garlic (*Allium sativum* L.) with tobacco chitinase and glucanase genes for tolerance to the fungus *Sclerotium cepivorum.* Afr J Biotechnol 12(22):3482–3492 https://doi.org/10.5897/ajb2013.12056
Francois LE (1994) Yield and quality response of salt-stressed garlic. Hort Sci 29:1314–1317
Fritsch R (2001) Taxonomy of the genus *Allium*: Contribution from IPK Gatersleben. Herbertia 56:19–50
García-Lampasona S, Asprelli P, Burba JL (2012) Genetic analysis of a garlic (*Allium sativum* L.) germplasm collection from Argentina. Sci Hort 138:183–189
Gore MA, Wright MH, Ersoz ES, Bouffard P, Szekeres ES, Jarvie TP, Hurwitz BL, Narechania A, Harkins TT, Grills GS, Ware DH, Buckler ES (2009) Large-scale discovery of gene enriched SNPs. Plant Genome 2:121–133
Haque MS, Hattori K (2017) Detection of viruses of Bangladeshi and Japanese garlic and their elimination through root meristem culture. Progressive Agric 28:55–63
Havey MJ, Ahn YK (2016) Single nucleotide polymorphisms and indel markers from the transcriptome of garlic. J Amer Soc Hort Sci 141(1):62–65
Hedrick UP (1972) Sturtevant's Edible Plants of the World. Dover Publications. ISBN0-486-20459-6
Hiei Y, Komari T, Kubo T (1997) Transformation of rice mediated by *Agrobacterium tumefaciens*. Plant Mol Biol 35:1–2
Hirata S, Abdelrahman M, Yamauchi N, Shigyo M (2016a) Diversity evaluation based on morphological, physiological and isozyme variation in genetic resources of garlic (*Allium sativum* L.) collected worldwide. Genes Genet Syst 91:161–173
Hirata S, Abdelrahman M, Yamauchi N, Shigyo M (2016b) Characteristics of chemical components in genetic resources of garlic *Allium sativum* collected from all over the world. Genet Resour Crop Evol 63:35–45
Hornickova J, Velisek J, Ovesna J, Stavelikova H (2009) Distribution of S-alk(en)yl-L-cysteine sulfoxides in garlic (*Allium sativum* L.). Czech J Food Sci 27:232–235
Insunza V, Valenzuela A (1995) Control of *Ditylenchus dipsaci* on garlic (*Allium sativum*) with extracts of medicinal plants from Chile. Nematropica 25:35–41
Ipek M, Ipek A, Simon PW (2003) Comparison of AFLPs, RAPD markers, and isozymes for diversity assessment of garlic and detection of putative duplicates in germplasm collections. J Amer Soc Hort Sci 128:24–252
Ipek M, Ipek A, Almquist SG, Simon PW (2005) Demonstration of linkage and development of the first low-density genetic map of garlic based on AFLP markers. Theor Appl Genet 110:22–236
Ipek M, Ipek A, Simon PW (2008) Rapid characterization of garlic clones with locus-specific DNA markers. Turk J Agri For 32:357–362

Ipek M, Sahin N, Ipek A, Cansev A, Simon PW (2015) Development and validation of new SSR markers from expressed regions in the garlic genome. Sci Agri 72:41–46. https://doi.org/10.1590/0103-9016-2014-0138

Jabbes N, Geoffriau E, Le Clerc V, Dridi B, Hannechi C (2011) Inter simple sequence repeat fingerprints for assess genetic diversity of Tunisian garlic populations. J Agri Sci 3:77–85

Jardinaud MF, Souvre A, Alibert G (1993) Transient GUS gene expression in *Brassica napus* electroporated microspores. Plant Sci 93:177–184

Jo M, Ham I, Moe K, Kwon S, Lu F, Park Y, Kim W, Won M, Kim T, Lee E (2012) Classification of genetic variation in garlic (*Allium sativum* L.) using SSR markers. Aust J CropSci 6:625–631

Jones HA, Mann LK (1963) Onions and Their Allies. Leonard Hill Books, London

Jones MG, Hughes J, Tregova A, Milne J, Tomsett AB, Collin HA (2004) Biosynthesis of the flavour precursors of onion and garlic. J Exp Bot 55(404):1903–1918

Kamenetsky R (1993) A living collection of *Allium* in Israel—problems of conservation and use. Diversity 9:24–26

Kamenetsky R (2007) Garlic: botany and horticulture. Hort Rev 33:123–171

Kamenetsky R, Rabinowitch DH (2001) Floral development in bolting garlic. Sexual Plant Reprod 13:23–241

Kamenetsky R, Rabinowitch HD (2002) Florogenesis. In: Rabinowitch HD, Currah L (eds) Allium Crop Sciences: Recent Advances. CAB International, Wallingford, UK, pp 31–57

Kamenetsky R, London Shafir I, Baizerman M, Khassanov F, Kik C, Rabinowitch HD (2004) Garlic (*Allium sativum* L.) and its wild relatives from Central Asia: evaluation for fertility potential. Acta Hort 637:83–91

Kamenetsky R, London Shafir I, Khassanov F, Kik C, van Heusden AW, Vrielink-van Ginkel M, Burger-Meijer K, Auger J, Arnault I, Rabinowitch HD (2005a) Diversity in fertility potential and organo-sulphur compounds among garlics from Central Asia. Biodivers Conserv 14:281–295

Kamenetsky R, London ShafirI, Khassanov F, Kik C, Van Heusden AW, Vrielink-Van Ginkel M, Burger-Meijer K, Auger J, Arnault I, Rabinowitch HD (2005b) Diversity in fertility potential and organo-sulphur compounds among garlics from Central Asia. Biodivers Conserv 14(2): 281–295.

Kamenetsky R, Faigenboim A, Mayer E, Michael T, Gershberg Ch, Kimhi S, Esquira I, Shalom S, Eshe D, Rabinowitch HD, ShermanA (2015) Integrated transcriptome catalogue and organ-specific profiling of gene expression in fertile garlic (*Allium sativum* L.). BMC Genomics 16:12

Kehr AE, Schäffer GW (1976) Tissue culture and differentiation in garlic. HortScience 11:422–423

Keller ERJ (2002) Cryopreservation of *Allium sativum* L. (Garlic). In: Towill LE, Bajaj YPS (eds) Cryopreservation of Plant Germplasm, vol 2. Springer, Berlin Heidelberg, Germany, pp 37–47

Keller ERJ, Senula A (2001) Progress in structuring and maintaining the garlic (*Allium sativum*) diversity for the European genres project. Acta Hort 555:189–193

Keller ERJ, Schubert L, Fuchs J (1996) Interspecific crosses of onion with distant *Allium* species and characterization of the presumed hybrids by means of flow cytometry, karyotype analysis and genomic *in situ* hybridization. Theor Appl Genet 92:417–424

Kenel F, Eady C, Brinch S (2010) Efficient *Agrobacterium tumefaciens*-mediated transformation and regeneration of garlic (*Allium sativum*) immature leaf tissue. Plant Cell Rep 29:223–230

Keurentjes JJ, Koornneef M, Vreugdenhil D (2008) Quantitative genetics in the age of omics. Curr Opin Plant Biol 11:123–128

Khanna HK, Raina SK (1999) *Agrobacterium* mediated transformation of Indica rice cultivars using binary and superbinary vectors. Aust J Plant Physiol 26:311–324

Khar A (2012) Cross amplification of onion derived microsatellites and mining of garlic ESTdatabase for assessment of genetic diversity in garlic. Acta Hort 969:289–295

Khar A, Yadav RC, Yadav N, Bhutáni RD (2005) Transient *gus* expression studies in onion (*Allium cepa* L.) and garlic (*Allium sativum* L.). Akdeniz Universitesi Ziraat Fakultesi Dergisi 18:301–304

Khar A, Asha Devi A, Lawande KE (2008) Analysis of genetic relationships among Indian garlic (*Allium sativum* L.) cultivars and breeding lines using RAPD markers. Indian J Genet 68:52–57

Kim DW, Jung TS, Nam SH, Kwon HR, Kim A, Chae SH, Choi SH, Kim DW, Kim RN, Park HS (2009) GarlicESTdb: an online database and mining tool for garlic EST sequences. BMC Plant Biol 9(1):61

King AM, Adams MJ, Lefkowitz E J, Carstens EB (Eds) (2012) Virus taxonomy: classification and nomenclature of viruses: ninth report of the international committee on taxonomy of viruses. Elsevier

Kondo T, Hasegawa H, Suszuki M (2000) Transformation and regeneration of garlic (*Allium sativum* L.) by *Agrobacterium*-mediated gene transfer. Plant Cell Rep 19:989–993

Kuhl JC, Cheung F, Yuan Q, Martin W, Zewdie Y, McCallum J, Catanach A, Rutherford P, Sink KC, Jenderek M, Prince JP, Town CD, Havey MJ (2004) A unique set of 11,008 onion (*Allium cepa*) ESTs reveals expressed sequence and genomic differences between monocot orders Asparagales and Poales. Plant Cell 16:114–125

Lafarge T, Bueno C, Frouin J, Jacquin L, Courtois B, Ahmadi N (2017) Genome-wide association analysis for heat tolerance at flowering detected a large set of genes involved in adaptation to thermal and other stresses. PLoS ONE 12:e0171254

Lamichhane JR, Barzman M, Booij K, Boonekamp P, Desneux N, Huber L, Kudsk P, Langrell SR, Ratnadass A, Ricci P, Sarah JL (2015) Robust cropping systems to tackle pests under climate change. A review. Agron Sustain Dev 35(2):443–459

Lampasona GS, Martınez L, Burba JL (2003) Genetic diversity among selected Argentinean garlic clones (*Allium sativum* L.) using AFLP (Amplified Fragment Length Polymorphism). Euphytica 132:115–119

Liu QQ, Zhang JL Wang ZY, Hong MM, Gu MH (1998) A highly efficient transformation system mediated by *Agrobacterium tumefaciens* in rice (*Oryza sativa* L.). Acta Phytophysiol Sin 24:259–271

Lu X, Ross CF, Powers JR, Aston DE, Rasco BA (2011) Determination of total phenolic content and antioxidant activity of garlic (*Allium sativum*) and elephant garlic (*Allium ampeloprasum*) by attenuated total reflectance-fourier transformed infrared spectroscopy. J Agri Food Chem 59:5215–5221

Ma Y, Wang HL, Zhang CJ, Kang YQ (1994) High rate of virus free plantlet regeneration via garlic scape tip culture. Plant Cell Rep 11:65–68

Ma KH, Gwag JG, Zhao WG, Dixit A, Lee GA, Kim HH, Chung IM, Kim NS, Lee JS, Ji JJ (2009) Isolation and characteristics of eight novel polymorphic microsatellite loci from the genome of garlic (*Allium sativum* L.). Sci Hort 122:355–361

Maas EV, Hoffman GJ (1977) Crop salt tolerance—current assessment. J Irrig Drain Eng 103:115–134

Maaß HI, Klaas M (1995) Intraspecific differentiation of garlic (*Allium sativum* L.) by isozyme and RAPD markers. Theor Appl Genet 91:89–97

Mangal JL, Singh RK, YadavAC Lal S, Pandey UC (1990) Evaluation of garlic cultivars for salinity tolerance. J Hort Sci 65(6):657–658

Manolio TA (2010) Genome wide association studies and assessment of the risk of disease. N Engl J Med 363:166–176

Martin WJ, McCallum J, Shigyo M, Jakse J, Kuhl JC, Yamane N, Pither-Joyce M, Gokce AF, Sink KC, Town CD, Havey MJ (2005) Genetic mapping of expressed sequences in onion and *in silico* comparisons with rice show scant colinearity. Mol Genet Genom 274:197

Mishra RK, Jaiswal RK, Kumar D, Saabale PR, Singh A (2014) Management of major diseases and insect pests of onion and garlic: a comprehensive review. J Plant Breed Crop Sci 6(11):160–170

Mostafa A, Sudisha J, El-Sayed M, Ito SI, Ikeda T, Yamauchi N, Shigyo M (2013) Aginsodie saponin a potent antifungal compound, and secondary metabolite analyses from *Allium nigrum* L. Phytochem Lett 6:274–280

Mousavi-Derazmahalleh M, Bayer PE, Hane JK, Babu V, Nguyen HT, Nelson MN, Erskine W, Varshney RK, Papa R, Edwards D (2018) Adapting legume crops to climate change using genomic approaches. Plant, Cell Environ 42:6–19

Moyer S (1996) Garlic in health history and world cuisine. Suncoast Press, St. Petrsberg, FL, pp 1–36

Nabulsi I, Al-Safadi B, Ali NM, Arabi MIE (2001) Evaluation of some garlic (*Allium sativum* L.) mutants resistant to white rot disease by RAPD analysis. Ann Appl Biol 138(2): 197–202

Nanda S, Chand SK, Mandal P, Tripathy P, Joshi RK (2016) Identification of novel source of resistance and differential response of *Allium* genotypes to purple blotch pathogen, *Alternaria porri* (Ellis) Ciferri. Plant Pathol J 32(6):519

Newman T, de Bruijin FJ, Green P, Keegstra K, Kende H, McIntosh L, Ohlrogge J, Raikhel N, Somerville S, Thomashow M, Retzel E, Somerville C (1994) Genes galore: a summary of methods for accessing results from large-scale partial sequencing of anonymous *Arabidopsis* cDNA clones. Plant Physiol 106:1241–1255

Novak FJ (1990) *Allium* tissue culture. In: Rabinowitch HD, Brewster JL (eds) Onions and allied crops, Vol II. CRC Press, Boca Raton, FL, USA, pp 233–250

Novák FJ (1980) Phenotype and cytological status of plants regenerated from callus cultures of *Allium sativum* L. Z Pflanzenzeucht 84:250

Ovesna J, Kucera L, Hornickova J, Svobodova L, Stavelikova H, Velisek J, Milella L (2011) Diversity of S-alk(en)yl cysteine sulphoxide content within a collection of garlic (*Allium sativum* L.) and its association with the morphological and genetic background assessed by AFLP. Sci Hort 129:541–547

Ovesná J, Leišová-Svobodová L, Kučera L (2014) Microsatellite analysis indicates the specific genetic basis of Czech bolting garlic. Czech J Genet Plant Breed 50:226–234

Panse R, Jain PK, Gupta A, Sasode DS (2013) Morphological variability and character association in diverse collection of garlic germplasm. Afr J Agri Res 8(23):2861–2869

Park MY, Yi NR, Lee HY, Kim ST, Kim M, Park JH, Kim JK, Lee JS, Cheong JJ, Choi YD (2002) Generation of chlorsulfuron-resistant transgenic transgenic garlic plants (*Allium sativum* L.) by particle bombardment. Mol Breed 9:171–181

Peffley EB, Hou A (2000) Bulb-type onion introgressants possessing *Allium fistulosum* L. genes recovered from interspecific hybrid backcrosses between *A. cepa* L. and *A. fistulosum* L. Theor Appl Genet 100:528–534

Peña-Iglesias A, Ayuso P (1982) Characterization of Spanish garlic viruses and their elimination by *in vitro* shoot apex culture. Acta Hort 127:183–193

Pooler MR, Simon PW (1993a) Characterization and classification of isozyme and morphological variation in a diverse collection of garlic clones. Euphytica 68:121–130

Pooler MR, Simon PW (1993b) Garlic flowering in response to clone, photoperiod, growth temperature and cold storage. HortScience 28:1085–1086

Porter DR, Jones HA (1932) Resistance of some of the cultivated species of *Allium* to pink root (*Phoma terrestris*). Phytopathology 23:290–298

Rabinowitch HD, Zeltzer O (1984) Collection, preservation, characterization and evaluation of *Allium* species growing wild in Israel: Selected Examples. Eucarpia, 3rd*Allium* Symposium, Wageningen, The Netherlands. Sept 1984, pp 27–36

Ramírez-Malagón R, Pérez-Moreno L, Borodanenko A, Salinas-González GJ, Ochoa-Alejo N (2006) Differential organ infection studies, potyvirus elimination, and field performance of virus-free garlic plants produced by tissue culture. Plant Cell Tiss Org Cult 86:103–110

Raza A, Razzaq A, Mehmood SS, Zou X, Zhang X, Lv Y, Xu J (2019) Impact of climate change on crops adaptation and strategies to tackle its outcome: a review. Plants 8(2):34

Reddy KR, Hodges HF, Kimball BA (2000) Crop ecosystem responses to global climate change: cotton. In: Reddy KR, Hodges HF (eds) Climate change and global crop productivity. CAB International, Wallingford, UK, pp 162–187

Robert U, Zel J, Ravnikar M (1998) Thermotherapy in virus elimination from garlic: influences on shoot multiplication from meristems and bulb formation *in vitro*. Sci Hort. 73:193–202

Robinson RA (2007) Self-Organizing Agroecosystems. Sharebooks Publishing, ISBN 6980-9783634-1-3

Robledo-Paz A, Cabrera Ponce JL, Villalobos Arámbula VM, Herrera Estrella L, Jofre Garfias AE (2004) Genetic transformation of garlic (*Allium sati*vum L.) by particle bombardment. HortScience 39:1208–1211

Rout E, Nanda S, Nayak S, Joshi RK (2014) Molecular characterization of NBS encoding resistance genes and induction analysis of a putative candidate gene linked to *Fusarium* basal rot resistance in *Allium sativum*. Physiol Mol Plant Pathol 85:15–24

Rout E, Nanda S, Joshi RK (2016) Molecular characterization and heterologous expression of a pathogen induced PR5 gene from garlic (*Allium sativum* L.) conferring enhanced resistance to necrotrophic fungi. Eur J Plant Pathol 144(2):345–360

Roy SJ, Tucker EJ, Tester M (2011) Genetic analysis of abiotic stress tolerance in crops. Curr Opin Plant Biol 14:232–239

Rubatzky VE, Yamaguchi M (1997) World vegetables: principles, production and nutritive values, 2nd edn. Chapman and Hall, New York

Sako I, Nakasome W, Okada K, Ohki S, Osaki T, Inouye T (1991) Yellow streak of rakkyo (*Allium chinense* G. Don). A newly recognized disease caused by garlic latent virus and onion yellow dwarf virus. Ann Phytopathol Soc Jpn 57:65–69

Sandhu SS, Brar PS, Dhall RK (2015) Variability of agronomic and quality characteristics of garlic (*Allium sativum* L.) ecotypes. SABRAO J Breed Genet 47(2):133–142

Sawahel WA (2002) Stable genetic transformation of garlic plants using particle bombardment. Cell Mol Biol Lett 7:49–59

Schwartz H (2004) Botrytis, downy mildew and purple blotch of onion. Colorado State University Cooperative Extension No. 2.941

Schwartz HF, Mohan SK (1995) Infectious biotic diseases. White Rot. In Mohan SK, Schwartz HF (eds) Compendium of onion and garlic diseases. American Phytopathological Society, pp 7–15

Shaaf S, Sharma R, Kilian B, Walther A, Özkan H, Karami E, Mohammadi B (2014) Genetic structure and eco-geographical adaptation of garlic landraces (*Allium sativum* L.) in Iran. Genet Resour. Crop Evol. https://doi.org/10.1007/s10722-014-0131-4

Silenzi JC, Moreno AM, Lucero JC (1985) Effect of irrigation with saline water on sprouting of cloves of garlic cv. Colorado. IDIA No. 433–436, 17–21 (Horticultural Abstracts, 56, 4145)

Son JH, Park KC, Lee S, Kim HH, Kim JH, Kim SH, Kim NS (2012) Isolation of cold-responsive genes from garlic, *Allium sativum*. Genes Genom 34:93–101. https://doi.org/10.1007/s13258-011-0187-x

Songstad DD, Somers DA, Griesbach RJ (1995) Advances in alternative DNA delivery techniques. Plant Cell Tiss Org. Cult 40:1–15

Stinchcombe JR, Hoekstra HE (2008) Combining population genomics and quantitative genetics: finding the genes underlying ecologically important traits. Heredity 100:158

Sugimoto H, Tsuneyoshi T, Tsukamoto M, Uragami Y, Etoh T (1991) Embryo-cultured hybrids between garlic and leek. Allium Improv Newsl 1:67–68

Sun X, Zhou S, Meng F, Liu S (2012) *De novo* assembly and characterization of the garlic (*Allium sativum*) bud transcriptome by Illumina sequencing. Plant Cell Rep 31:1823–1828

Sun X, Ma GQ, Cheng B, Li H, Liu SQ (2013) Identification of differentially expressed genes in shoot apex of garlic (*Allium sativum* L.) using Illumina sequencing. J Plant Stud 2:136

Takagi H (1990) Garlic *Allium sativum* L. In: Brewster JL, Rabinowitch HD (eds) Onion and allied crops, vol III. Biochemistry, food science and minor crops. CRC Press, Boca Raton, FL, pp 109–146

Thoen MP, Davila Olivas NH, Kloth KJ, Coolen S, Huang PP, Aarts MG, Bac-Molenaar JA, Bakker J, Bouwmeester HJ, Broekgaarden C (2017) Genetic architecture of plant stress resistance: multi-trait genome-wide association mapping. New Phytol 213:1346–1362

Ucman R, Zel J, Ravnikar M (1998) Thermotherapy in virus elimination from garlic: influences on shoot multiplication from meristems and bulb formation *in vitro*. Sci Hort. 73(4):193–202

Valdez JG, Makuch MA, Ordovini AF, Masuelli RW, Overy DP, Piccolo RJ (2006) First report of *Penicillium allii* as a field pathogen of garlic (*Allium sativum*). Plant Pathol 55(4):583

Vidal DBC, Mello MLS, Liig D (1993) Chromosome number and DNA content in cells of a biotechnologically selected somaclone of garlic (*Allium sativum* L.). Rev Brasil Genet 16:347–356

Vieira RL, da Silva AL, Zaffari GR, Steinmacher DA, de Freitas Fraga HP, Guerra MP (2015) Efficient elimination of virus complex from garlic (*Allium sativum* L.) by cryotherapy of shoot tips. Acta Physiol Plant 37:1733

Volk GM, Henk AD, Richards CM (2004) Genetic diversity among U.S. garlic clones as detected using AFLP methods. J Amer Soc Hort Sci 129:559–569

Wang H, Li X, Liu X, Oiu Y, Song J, Zhang X (2016) Genetic diversity of garlic (*Allium sativum* L.) germplasm from China by fluorescent-based AFLP, SSR and InDel markers. Plant Breed. 135:743–750. https://doi.org/10.1111/pbr.12424

Wani SH, Choudhary M, Kumar P, Akram NA, Surekha C, Ahmad P, Gosal SS (2018) Marker-assisted breeding for abiotic stress tolerance in crop plants. In: Gosal SS, Wani SH (eds) Biotechnologies of crop improvement, vol 3. Springer. Berlin, Heidelberg, Germany, pp 1–23

Wei NS, We YF (1992) Identification of virus diseases and virus free meristem culture of garlic. Acta Univ Agri Bor Occid 20(1):76–81

Wu M, Jin F, Zhang J, Yang L, Jiang D, Li G (2012) Characterization of a novel bipartite double-stranded RNA mycovirus conferring hypovirulence in the pathogenic fungus *Botrytis porri*. J Virol 86:6605–6619

Wu C, Wang M, Dong Y, Cheng Z, Meng H (2015) Growth, bolting and yield of garlic (*Allium sativum* L.) in response to clove chilling treatment. Sci Hort 194:43–52

Wu C, Wang M, Cheng Z, Meng H (2016) Response of garlic (*Allium sativum* L.) bolting and bulbing to temperature and photoperiod treatments. Biol Open 5(4):507–18. https://doi.org/10.1242/bio.016444

Xue HM, Araki H, Shi L, Yakuwa T (1991) Somatic embryogenesis and plant regeneration in basal plate- and receptacle-derived callus cultures garlic (*Allium sativum* L.). J Jpn Soc Hort Sci 60:627–634

Yanagino T, Sugawara E, Watanabe M, Takahata Y (2003) Production and characterization of an interspecific hybrid between leek and garlic. Theor Appl Genet 107(1):1–5

Zewde T, Fininsa C, Sakhuja PK, Ahmed S (2007) Association of white rot (*Sclerotium cepivorum*) of garlic with environmental factors and cultural practices in the North Shewa highlands of Ethiopia. Crop Protec 26: 1566e1573

Zewdie Y, Havey MJ, Prince JP, Jenderek MM (2005) The first genetic linkages among expressed regions of the garlic genome. J Amer Soc Hort Sci 130(4):569–574

Zhao WG, Chung JW, Lee GA, Ma KH, Kim HH, Kim KT, Chung IM, Lee JK, Kim NS, Kim SM, Park YJ (2011) Molecular genetic diversity and population structure of a selected core set in garlic and its relatives using novel SSR markers. Plant Breed 130:46–54

Zheng SJ, Henken B, Ahn YK, Krens FA, Kik C (2004) The development of a reproducible *Agrobacterium tumefaciens* transformation system for garlic (*Allium sativum* L.) and the production of transgenic garlic resistant to beet armyworm (*Spodoptera exigua* Hübner). Mol Breed 14:293–307

Zilberman D, Lipper L, McCarthy N, Gordon B (2018) Innovation in response to climate change. In: Lipper L, McCarthy N, Zilberman D, Asfaw S, Branca G (eds) Climate smart agriculture. Springer, Cham, Switzerland, pp 49–74